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Effects of urease inhibitors, nitrification inhibitors and pesticides on denitrification in soil

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INTRODUCTION

World consumption of fertilizer nitrogen has increased at a phenomenal rate since World War II. It will continue to increase because the international need for food and fiber cannot be met without increased use of fertilizer nitrogen. This trend will result in increased levels of nitrogen in soils, natural waters, crop residues and municipal and agricultural wastes, and there is national and international concern about its potential adverse effects on environmental quality and public health.

The overall objective of fertilizer nitrogen research is to maximize the efficiency of plant use of the applied nitrogen. Any increase in this efficiency will increase the agronomic and economic value of fertilizer as a means of increasing crop production, conserve energy and the raw materials needed to make nitrogen fertilizers, and minimize possible adverse effects on the environment that may result from inefficient nitrogen use (Bremner and Hauck, 1974). Nitrogen use efficiency can be increased by improving plant use of nitrogen and by reducing losses of plant-available nitrogen by leaching, denitrification and other processes.

One approach to increasing the efficiency of nitrogen use by crops is to find methods of inhibiting the nitrogen transformations in soils that lead to losses of plant-available nitrogen. The growing importance of urea fertilizer in world agriculture has stimulated extensive research to find compounds that will retard hydrolysis of urea to ammonia by soil

urease and thereby reduce ammonia volatilization and other problems encountered in the use of this fertilizer. This research has shown that several compounds (notably certain phosphoroamides, quinones and polyhydric phenols) have potential value for retarding urea hydrolysis in soil, and there is a clear need for information concerning the effects of these compounds on other nitrogen transformations in soil. Several urease inhibitors have been tested for their effects on nitrification in soil (Bundy and Bremner, 1974a), but no studies of the effects of urease inhibitors on denitrification in soil have been reported.

One of the most promising approaches to increasing the recovery of fertilizer nitrogen by crops and to reducing the potential environmental problems associated with the use of nitrogen fertilizers is to find compounds that will effectively inhibit oxidation of ammonium to nitrate by the nitrifying organisms in soil. This approach has received considerable attention during the past 20 years and numerous compounds, notably nitrapyrin (N-Serve) and etridiazole (Dwell), have been patented for inhibition of nitrification in soil. Studies of the effects of some of these nitrification inhibitors on urease activity in soil have been reported (Bundy and Bremner, 1974b; Bremner and Bundy, 1976), but very little information is available concerning the effects of nitrification inhibitors on denitrification in soil.

The international need for food and fiber cannot be met without increased use of pesticides as well as nitrogen fertilizers because pesticides are vitally important to increasing and protecting the quantity and quality of food commodities. Herbicides, insecticides and

fungicides are used in agriculture because they exhibit some degree of toxicity to specific groups of weeds, insects and plant pathogens. Pesticide use in agriculture is subject to state and federal regulations designed to protect public health and environmental quality. However, a current concern of the regulatory agencies is the potential adverse effects of pesticides on soil microbial processes, especially those involving nitrogen transformations. Continuing research is needed to better understand and quantify the extent to which pesticides influence nitrogen transformations in soil.

Many pesticides enhance mineralization of soil organic nitrogen, apparently because they destroy a part of the soil population that then decays to release mineral nitrogen, but most pesticides have little if any influence on N_2-fixing organisms when applied at recommended rates (Goring and Laskowski, 1982). Most pesticides thus far studied for their effects on nitrogen transformations in soil either inhibit nitrification or have no effect on nitrification. Inhibition of nitrification has significant agronomic impacts. It can increase the amount of NH_4^+ available to plants, which may or may not be beneficial, and it can reduce losses of nitrogen from soil via leaching and denitrification. These agronomic effects need to be considered when pesticides that are known to inhibit nitrification are utilized. Similar consideration of agronomic impacts should be given to pesticides that have the potential to reduce denitrification, but very few pesticides have been studied for their effects on denitrification.

The purpose of the work reported in this dissertation was to

determine the effects of various urease inhibitors, nitrification inhibitors and pesticides on denitrification of nitrate in soil. The urease and nitrification inhibitors tested included those considered to be the most effective compounds currently available for retardation of urea hydrolysis and nitrification in soil. The pesticides tested included herbicides, insecticides and fungicides commonly used for pest control in Iowa.

PART I. EFFECTS OF UREASE INHIBITORS ON DENITRIFICATION IN SOIL

INTRODUCTION

The rapidly increasing importance of urea as a nitrogen fertilizer in world agriculture has stimulated research to find compounds that will retard hydrolysis of fertilizer urea by soil urease and thereby reduce the problems encountered in the use of this fertilizer (Bremner and Douglas, 1971, 1973; Bundy and Bremner, 1973a; Martens and Bremner, 1984). This research has shown that several compounds (notably certain quinones, polyhydric phenols and phosphoroamides) have potential value for retardation of urea hydrolysis in soil, and there is a clear need for information concerning the effects of these compounds on other nitrogen transformations in soils. Studies of the effects of some of these urease inhibitors on nitrification in soils have been reported (Bundy and Bremner, 1974a), but no information is available concerning their effects on denitrification in soils.

I report here the results of studies of the effects of 14 urease inhibitors on denitrification of nitrate in soils. The urease inhibitors studied [catechol, hydroquinone, 1,4-benzoquinone, 2,5-dichloro-1,4-benzoquinone, 2,6-dichloro-1,4-benzoquinone, 2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, sodium-4-chloromercuri-benzoate, phenylphosphorodiamidate, *N*-phenylphosphoric triamide, phosphoric triamide, *N*-(diaminophosphinyl)benzeneacetamide, *N*-(diaminophosphinyl)benzamide and 4-fluoro-*N*-(diaminophosphinyl)-benzamide] were those found most effective in research to evaluate

various compounds as inhibitors of urea hydrolysis in soils (Bremner and Douglas, 1971; Bundy and Bremner, 1973a; Martens and Bremner, 1984).

MATERIALS AND METHODS

The soil used was a surface (0-15 cm) sample of a typic Haplaquoll of the Webster series. Analyses performed as described by Zantua and Bremner (1975) showed that it had a pH of 7.5 and contained 3.3% organic carbon, 30% clay, 39% silt and 31% sand. The soil was sieved (<2 mm) in the field-moist condition, and subsamples of the sieved soil were air-dried or stored at 5°C.

The names and sources of the 14 urease inhibitors studied and references to use of these compounds for inhibition of soil urease activity are reported in Table 1. To study the effects of these inhibitors on denitrification of nitrate in soil, samples of air-dried or field-moist Webster soil containing 30 g of oven-dry material were placed in 1.2-L flasks fitted with standard taper (34/45) ground-glass joints and treated with 5 mL of H_2O containing 0, 0.03, 0.3 or 1.5 mg of the inhibitor under study, 5 mL of H_2O containing 9 mg N as KNO_3 and 0 or 4.5 mg of C as mannitol and the amount of H_2O needed to bring the total volume of H_2O to 15 mL. Each flask was then sealed with a glass stopper fitted with a standard taper (34/45) ground-glass joint and a glass stopcock and was connected to a manifold system attached to a mercury (Hg) manometer. The flasks were evacuated via the stopcock and filled with helium (He) to slightly above atmospheric pressure, and this process was repeated three times. The flasks were then brought to atmospheric pressure with He and placed in an incubator at 30°C. The atmospheres in the flasks were subsequently sampled at intervals for determination of

Table 1. Urease inhibitors studied

Name	Source[a]	Reference[b]
1,2-Dihydroxybenzene (catechol)	F	1
1,4-Dihydroxybenzene (hydroquinone)	F	1
1,4-Benzoquinone	F	1
2,5-Dichloro-1,4-benzoquinone	E	1
2,6-Dichloro-1,4-benzoquinone	E	1
2,5-Dimethyl-1,4-benzoquinone	E	1
2,6-Dimethyl-1,4-benzoquinone	I	1
Sodium-4-chloromercuribenzoate	S	1
Phenylphosphorodiamidate	I	2-6
N-Phenylphosphoric triamide	N	4
Phosphoric triamide	A	4
N-(Diaminophosphinyl)-benzeneacetamide	N	4
N-(Diaminophosphinyl)benzamide	N	4
4-Fluoro-*N*-(Diaminophosphinyl)-benzamide	N	4

[a] F, Fisher Scientific Co., Fair Lawn, NJ; E, Eastman Organic Chemicals, Rochester, NY; I, ICN Pharmaceuticals Inc., Plainview, NY; S, Sigma Chemical Co., St. Louis, MO; A, Allied Corporation, Solvay, NY; N, Norwich-Eaton Pharmaceuticals, Norwich, NY.

[b] 1, Bremner and Douglas (1971); 2, Byrnes et al. (1983); 3, Held et al. (1976); 4, Martens and Bremner (1984); 5, Matzel et al. (1979); 6, Vlek et al. (1980).

N_2O, NO, N_2 and O_2 by the GC-ultrasonic detector method described by Blackmer and Bremner (1977). The analyses for O_2 were performed to check that anaerobic conditions had been maintained. The results of analyses for NO are not reported because only trace amounts of this gas could be detected. All experiments were performed in triplicate.

Analyses of soil samples for nitrite were performed by the colorimetric procedure described by Bremner (1965). Analyses for nitrate were performed by the steam distillation method described by Bremner and Keeney (1966).

Preliminary work showed that no loss of nitrate or production of nitrite, N_2O, NO or N_2 could be detected when soil samples previously sterilized by autoclaving at 121°C for 1 hour were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg nitrate-N as KNO_3.

RESULTS AND DISCUSSION

None of the urease inhibitors studied had a significant effect on denitrification of nitrate by soil microorganisms when applied at the rate of 1 or 10 μg g^{-1} soil. The data obtained using 10 μg g^{-1} soil are reported in Table 2.

Three of the inhibitors (2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone and *N*-phenylphosphoric triamide) retarded denitrification when applied at the rate of 50 μg g^{-1} soil, but hydroquinone, 1,4-benzoquinone, 2,6-dichloro-1,4-benzoquinone, sodium-4-chloromercuribenzoate, phosphoric triamide, *N*-(diaminophosphinyl)-benzeneacetamide, *N*-(diaminophosphinyl)benzamide and 4-fluoro-*N*-(diaminophosphinyl)benzamide enhanced denitrification when applied at this rate (Table 3). Hydroquinone and 1,4-benzoquinone decreased the ratio of N_2/N_2O in the gaseous products of denitrification, whereas four of the phosphoroamides studied [phosphoric triamide, *N*-(diamino-phosphinyl)benzeneacetamide, *N*-(diaminophosphinyl)benzamide and 4-fluoro-*N*-(diaminophosphinyl)benzamide] increased this ratio (i.e., promoted microbial reduction of N_2O) (Table 3). Sodium-4-chloro-mercuribenzoate enhanced denitrification when applied at the rate of 50 μg g^{-1} soil, but it inhibited nitrite reduction and thereby decreased production of N_2O and N_2 (Table 3).

Table 4 shows the data obtained when the experiments using 50 μg inhibitor g^{-1} soil reported in Table 3 were repeated using soil amended with mannitol to promote denitrification of nitrate by soil

Table 2. Effects of 10 μg g^{-1} soil of various urease inhibitors on denitrification of nitrate in Webster soil[a]

Urease inhibitor	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	---------- μg g^{-1} soil ----------				
None	109	0	34	74	108
Catechol	108	0	35	74	109
Hydroquinone	109	0	34	74	108
1,4-Benzoquinone	109	0	34	74	108
2,5-Dichloro-1,4-benzoquinone	108	0	37	70	107
2,6-Dichloro-1,4-benzoquinone	108	0	35	72	107
2,5-Dimethyl-1,4-benzoquinone	107	0	34	73	107
2,6-Dimethyl-1,4-benzoquinone	107	0	35	72	107
Sodium-4-chloromercuribenzoate	110	0	33	76	109
Phenylphosphorodiamidate	109	0	32	76	108
N-Phenylphosphoric triamide	108	0	35	73	108
Phosphoric triamide	110	0	34	75	109
N-(Diaminophosphinyl)-benzeneacetamide	110	0	34	75	109
N-(Diaminophosphinyl)benzamide	110	0	34	76	110
4-Fluoro-*N*-(diaminophosphinyl)-benzamide	109	0	35	74	109

[a] Samples of air-dried soil containing 30 g of oven-dry material were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 0.3 mg of the inhibitor specified.

Table 3. Effects of 50 μg g^{-1} soil of various urease inhibitors on denitrification of nitrate in Webster soil[a]

Urease inhibitor	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	μg g^{-1} soil				
None	109	0	34	74	108
Catechol	106	0	34	71	105
Hydroquinone	122	3	62	59	124
1,4-Benzoquinone	121	0	61	61	122
2,5-Dichloro-1,4-benzoquinone	106	0	35	72	107
2,6-Dichloro-1,4-benzoquinone	115	0	42	75	117
2,5-Dimethyl-1,4-benzoquinone	82	22	2	59	83
2,6-Dimethyl-1,4-benzoquinone	85	31	3	52	86
Sodium-4-chloromercuribenzoate	151	85	8	58	151
Phenylphosphorodiamidate	107	0	15	93	108
N-Phenylphosphoric triamide	97	0	32	66	98
Phosphoric triamide	126	0	26	100	126
N-(Diaminophosphinyl)-benzeneacetamide	126	0	0	125	125
N-(Diaminophosphinyl)benzamide	128	0	9	120	129
4-Fluoro-*N*-(diaminophosphinyl)-benzamide	121	0	21	102	123

[a] Samples of air-dried soil containing 30 g of oven-dry material were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 1.5 mg of the inhibitor specified.

Table 4. Effects of 50 μg g^{-1} soil of various urease inhibitors on denitrification of nitrate in Webster soil amended with mannitol[a]

Urease inhibitor	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	μg g^{-1} soil				
None	278	203	66	9	278
Catechol	224	152	61	10	223
Hydroquinone	283	251	23	9	283
1,4-Benzoquinone	293	257	28	10	295
2,5-Dichloro-1,4-benzoquinone	264	211	47	8	266
2,6-Dichloro-1,4-benzoquinone	278	223	48	7	278
2,5-Dimethyl-1,4-benzoquinone	32	24	2	6	32
2,6-Dimethyl-1,4-benzoquinone	13	8	0	5	13
Sodium-4-chloromercuribenzoate	210	180	16	12	208
Phenylphosphorodiamidate	281	211	61	9	281
N-Phenylphosphoric triamide	279	203	64	12	279
Phosphoric triamide	282	194	77	13	284
N-(Diaminophosphinyl)-benzeneacetamide	274	202	61	10	273
N-(Diaminophosphinyl)benzamide	282	195	74	11	280
4-Fluoro-*N*-(diaminophosphinyl)-benzamide	274	198	65	11	274

[a] Samples of air-dried soil containing 30 g of oven-dry material were incubated (30°C, 15 mL of H_2O) under He for 1 day after treatment with 9 mg of nitrate-N as KNO_3, 4.5 mg of carbon as mannitol and 1.5 mg of the inhibitor specified.

microorganisms. The data reported show that 2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, catechol and sodium-4-chloromercuribenzoate retarded denitrification under these conditions and that hydroquinone, 1,4-benzoquinone, 2,5-dichloro-1,4-benzoquinone and 2,6-dichloro-1,4-benzoquinone inhibited nitrite reduction and decreased production of N_2O (Table 4).

The experiments reported in Tables 3 and 4 were performed with soil samples that were air-dried before use. Tables 5 and 6 show the results obtained when these experiments were repeated using samples of field-moist soil. The data in Table 5 show that two of the inhibitors (2,5-dichloro-1,4-benzoquinone and 2,6-dichloro-1,4-benzoquinone) retarded denitrification when applied at the rate of 50 μg g^{-1} soil and that seven of the inhibitors [hydroquinone, 2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, sodium-4-chloro-mercuribenzoate, phenylphosphorodiamidate, *N*-(diaminophosphinyl)-benzeneacetamide and *N*-(diaminophosphinyl)benzamide] enhanced denitrification when applied at this rate. Six of the inhibitors [hydroquinone, 2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, sodium-4-chloromercuribenzoate, phenylphosphorodiamidate and *N*-(diaminophosphinyl)benzeneacetamide] inhibited nitrite reduction when applied at the rate of 50 μg g^{-1} soil, and four of them (2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, sodium-4-chloromercuribenzoate and phenylphosphorodiamidate) also increased the ratio of N_2/N_2O in the gaseous products of denitrification when applied at this rate (Table 5).

Table 5. Effects of 50 μg g^{-1} soil of various urease inhibitors on denitrification of nitrate in Webster soil[a]

Urease inhibitor	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	---------- μg g^{-1} soil ----------				
None	51	0	20	31	51
Catechol	51	0	19	33	52
Hydroquinone	66	9	20	39	68
1,4-Benzoquinone	55	8	21	26	55
2,5-Dichloro-1,4-benzoquinone	42	0	16	25	41
2,6-Dichloro-1,4-benzoquinone	39	0	13	24	37
2,5-Dimethyl-1,4-benzoquinone	71	48	0	23	71
2,6-Dimethyl-1,4-benzoquinone	71	49	0	21	70
Sodium-4-chloromercuribenzoate	106	78	9	18	105
Phenylphosphorodiamidate	72	18	9	45	72
N-Phenylphosphoric triamide	44	0	20	26	46
Phosphoric triamide	46	0	18	29	47
N-(Diaminophosphinyl)-benzeneacetamide	77	11	27	39	77
N-(Diaminophosphinyl)benzamide	65	2	23	39	64
4-Fluoro-*N*-(diaminophosphinyl)-benzamide	48	0	19	27	46

[a] Samples of field-moist soil containing 30 g of oven-dry material were incubated (30°C, 15 mL of H_2O) under He for 7 days after treatment with 9 mg of nitrate-N as KNO_3 and 1.5 mg of the inhibitor specified.

Table 6 shows that, when the 14 urease inhibitors studied were applied at the rate of 50 μg g^{-1} soil to field-moist soil amended with mannitol, eight of them [catechol, 2,5-dichloro-1,4-benzoquinone, 2,6-dichloro-1,4-benzoquinone, 2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, phenylphosphorodiamidate, *N*-phenyl-phosphoric triamide and 4-fluoro-*N*-(diaminophosphinyl)benzamide] retarded denitrification. Catechol, 2,5-dichloro-1,4-benzoquinone and 2,6-dichloro-1,4-benzoquinone also decreased the N_2/N_2O ratio in the gaseous products of denitrification when applied at the rate of 50 μg g^{-1} soil, whereas 2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone and phenylphosphorodiamidate increased the N_2/N_2O ratio when applied at this rate (i.e., promoted reduction of N_2O by soil microorganisms). Hydroquinone, 1,4-benzoquinone and sodium-4-chloro-mercuribenzoate enhanced denitrification when applied at the rate of 50 μg g^{-1} soil, and hydroquinone and 1,4-benzoquinone decreased the N_2/N_2O ratio in the gaseous products of denitrification (i.e., inhibited reduction of N_2O by soil microorganisms) (Table 6). Sodium-4-chloro-mercuribenzoate promoted reduction of N_2O by soil microorganisms, but inhibited nitrite reduction when applied at the rate of 50 μg g^{-1} soil (Table 6).

To summarize, none of the urease inhibitors tested had a significant effect on denitrification of nitrate by soil microorganisms when applied at the rate of 1 or 10 μg g^{-1} soil. A few of the inhibitors (notably 2,5-dimethyl-1,4-benzoquinone and 2,6-dimethyl-1,4-benzoquinone) retarded denitrification when applied at the rate of 50 μg g^{-1} soil, but most of

Table 6. Effects of 50 μg g^{-1} soil of various urease inhibitors on denitrification of nitrate in Webster soil amended with mannitol[a]

Urease inhibitor	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	---------- μg g^{-1} soil ----------				
None	275	195	17	63	275
Catechol	249	167	26	56	249
Hydroquinone	302	207	59	36	302
1,4-Benzoquinone	293	204	58	31	293
2,5-Dichloro-1,4-benzoquinone	251	162	37	50	249
2,6-Dichloro-1,4-benzoquinone	251	162	28	61	251
2,5-Dimethyl-1,4-benzoquinone	235	187	2	46	235
2,6-Dimethyl-1,4-benzoquinone	220	172	5	42	219
Sodium-4-chloromercuribenzoate	299	257	3	40	300
Phenylphosphorodiamidate	255	174	9	72	255
N-Phenylphosphoric triamide	255	169	17	69	255
Phosphoric triamide	267	188	18	60	266
N-(Diaminophosphinyl)-benzenacteamide	265	180	13	70	263
N-(Diaminophosphinyl)benzamide	285	193	11	82	286
4-Fluoro-*N*-(diaminophosphinyl)-benzamide	252	172	13	67	252

[a] Samples of field-moist soil containing 30 g of oven-dry material were incubated (30°C, 15 mL of H_2O) under He for 3 days after treatment with 9 mg of nitrate-N as KNO_3, 4.5 mg of carbon as mannitol and 1.5 mg of the inhibitor specified.

them either had no significant effect on denitrification or enhanced denitrification when applied at this rate.

SUMMARY

The effects of 14 urease inhibitors on denitrification of nitrate in soil were studied by determining the effects of 1, 10 and 50 μg g^{-1} soil of each inhibitor on the amounts of nitrate lost and the amounts of nitrite, N_2O and N_2 produced when samples of soil were incubated anaerobically after treatment with nitrate or with nitrate and mannitol. The inhibitors used were catechol, hydroquinone, 1,4-benzoquinone, 2,5-dichloro-1,4-benzoquinone, 2,6-dichloro-1,4-benzoquinone, 2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, sodium-4-chloromercuribenzoate, phenylphosphorodiamidate, *N*-phenylphosphoric triamide, phosphoric triamide, *N*-(diaminophosphinyl)-benzeneacetamide, *N*-(diaminophosphinyl)benzamide and 4-fluoro-*N*-(diaminophosphinyl)benzamide.

None of the urease inhibitors studied had a significant effect on denitrification when applied at the rate of 1 or 10 μg g^{-1} soil. Three of the inhibitors (2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone and *N*-phenylphosphoric triamide) retarded denitrification when applied at the rate of 50 μg g^{-1} soil, but most of the other urease inhibitors tested enhanced denitrification when applied at this rate. Four of the inhibitors (2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, sodium-4-chloromercuribenzoate and catechol) retarded denitrification when applied at the rate of 50 μg g^{-1} soil to soils amended with mannitol.

PART II. EFFECTS OF NITRIFICATION INHIBITORS ON DENITRIFICATION IN SOIL

INTRODUCTION

Recent concern about losses of costly fertilizer nitrogen (N) by leaching and denitrification and pollution of surface and groundwaters by fertilizer-derived nitrate has stimulated international interest in research to find compounds that will reduce these problems by effectively inhibiting nitrification of fertilizer N in soil. Numerous studies to evaluate various compounds as soil nitrification inhibitors have been reported during the past 20 years, and many compounds, notably nitrapyrin and etridiazole, have been patented for inhibition of nitrification in soil (Hauck, 1980; 1984), but very little is known about the effects of these compounds on other transformations of N in soils. Studies of the effects of some of these compounds on urease activity in soils have been reported (Bundy and Bremner, 1974b; Bremner and Bundy, 1976), but very little information is available concerning the effects of nitrification inhibitors on denitrification of nitrate in soil.

Although there is very little literature concerning the effects of nitrification inhibitors on denitrification in soil, there are reports that small amounts of sodium azide (Mitsui et al., 1964), phenylmercuric acetate, potassium azide, 2-sulfanilamidothiazole (Henninger and Bollag, 1976), etridiazole (Mills and McElhannon, 1984) and nitrapyrin (Mitsui et al., 1964; Mills et al., 1976; McElhannon and Mills, 1981a; Mills and McElhannon, 1983; Mills, 1984) inhibit denitrification in soil. However, nitrapyrin has also been reported to enhance denitrification in sand (Notton et al., 1979) and to have little or no effect on denitrification

in soil (Bollag and Henninger, 1976; Bremner and Blackmer, 1980). Other nitrification inhibitors reported to have little or no effect on denitrification in soil are dicyandiamide (Mitsui et al., 1964), phenylmercuric acetate (Sandhu and Moraghan, 1972), 2-amino-4-chloro-6-methylpyrimidine, 4-amino-1,2,4-triazole, 2,5-dichloroaniline and 3'-chloracetanilide (Henninger and Bollag, 1976).

I report here the results of studies of the effects of 28 nitrification inhibitors on denitrification of nitrate in soil. The inhibitors studied included seven compounds which are produced commercially for use with nitrogen fertilizers. Four of these compounds (2-amino-4-chloro-6-methylpyrimidine, 2-mercaptobenzothiazole, sulfathiazole and thiourea) are produced and used mainly in Japan, one (dicyandiamide) is produced and marketed in both Japan and West Germany, and two (nitrapyrin and etridiazole) are licensed for use as nitrification inhibitors in the United States (Hauck, 1984). Nitrapyrin [2-chloro-6-(trichloromethyl)pyridine] is sold under the trade name of N-Serve (Dow Chemical U.S.A.). Etridiazole [5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole] is marketed under the trade name of Dwell (Olin Corporation). Nitrapyrin formulations include N-Serve 24, an oil-soluble formulation for use with anhydrous ammonia, dry ammonium, and urea fertilizers, and N-Serve 24E, an emulsifiable formulation for use with aqua ammonia and other liquid nitrogen fertilizers or with liquid animal wastes. The other compounds studied have been reported to inhibit nitrification in soil and some have been patented as nitrification inhibitors (see Bundy and Bremner, 1973b; Hauck, 1980).

MATERIALS AND METHODS

The soils used were surface (0-15 cm) samples of a typic Haplaquoll of the Canisteo series and of a typic Calciaquoll of the Harps series. Before use, they were air-dried and crushed to pass through a 2-mm screen. Analyses performed as described by Zantua and Bremner (1975) showed that the Canisteo soil had a pH of 7.5 and contained 3.3% organic carbon, 30% clay, 39% silt and 31% sand, and that the Harps soil had a pH of 7.9 and contained 6.6% organic carbon, 41% clay, 49% silt and 10% sand.

The names and sources of the 28 nitrification inhibitors studied and references to use of these compounds are reported in Table 7. To study the effects of these inhibitors on denitrification of nitrate in soil, 30-g samples of air-dried soil were placed in 1.2-L flasks fitted with standard taper (34/45) ground-glass joints and treated with 5 mL of H_2O containing 9 mg of N as KNO_3, 5 mL of H_2O containing 0, 0.3, 0.75, 1.5 or 3.0 mg of the inhibitor under study and 5 mL of H_2O containing 0 or 4.5 mg of C as mannitol. Each flask was then sealed with a glass stopper and fitted with a standard taper (34/45) ground-glass joint and a glass stopcock and was connected to a manifold system attached a mercury (Hg) manometer. The flasks were evacuated via the stopcock and filled with helium (He) to slightly above atmospheric pressure, and this process was repeated three times. The flasks were then brought to atmospheric pressure with He and placed in an incubator at 30°C. The atmospheres in the flasks were subsequently sampled at intervals for determination of

Table 7. Nitrification inhibitors studied

Name	Source[a]	Reference[b]
Nitrapyrin (N-Serve)	D	1-4
Potassium azide	E	1,5,6
2-Amino-4-chloro-6-methylpyrimidine	T	1,4
2-Mercaptobenzothiazole	A	1,4
Sulfathiazole	S	1,4
Etridiazole (Dwell)	O	7-10
4-Amino-1,2,4-triazole	I	1,4
3-Mercapto-1,2,4-triazole	I	1,4
2,4-Diamino-6-trichloromethyl-*s*-triazine	AM	1,4
Potassium amylxanthate	P	11
Potassium ethylxanthate	P	11-13
Sodium ethylxanthate	P	13
Sodium isopropylxanthate	P	11
Guanylthiourea	F	4
Thiourea	FS	4
2-Chloroacetamide	E	14
2-Fluoroacetamide	A	14
4-Nitrobenzotrichloride	PN	15
4-Mesylbenzotrichloride	PN	15
Caffeic acid	S	16,17
Chlorogenic acid	S	16,17
p-Coumaric acid	S	16
Ferulic acid	A	16,17
Gallic acid	F	18
Sodium thiocarbonate	E	8,19
Sodium diethyldithiocarbamate	E	1,12,13
Phenylmercuric acetate	S	1,20
Dicyandiamide	FS	1,4,21

[a] D, Dow Chemical Co., Midland, MI; E, Eastman Organic Chemicals, Rochester, NY; T, Toya Koatsu, Japan; A, Aldrich Chemical Co., Inc., Milwaukee, WI; S, Sigma Chemical Co., St. Louis, MO; O, Olin Corporation, Little Rock, AR; I, ICN Pharmaceuticals, Inc., Plainview, NY; AM, American Cyanamid Co., Princeton, NJ; P, Pfaltz and Bauer, Inc., Stamford, CT; F, Fairchild Chemical Co., Inc., Blythewood, SC; FS Fisher Scientific Co., Fair Lawn, NJ; PN, E. I. duPont de Nemours & Co., Wilmington, DE.

[b] 1, Bundy and Bremner (1973b); 2, Goring (1962a); 3, Goring (1962b); 4, Hauck (1972); 5, Hughes and Welch (1970); 6, Parr et al. (1971); 7, Malzer (1979); 8, Rodgers et al. (1983); 9, Sommer (1972); 10, Varsa and Huber (1983); 11, Ashworth et al. (1980); 12, Hooper and Terry (1973); 13, Lees (1952); 14, Frankenberger and Tabatabai (1982); 15, Heytler et al. (1984); 16, Lodhi (1982); 17, Rice and Pancholy (1974); 18, Rice and Pancholy (1973); 19, Ashworth et al (1977); 20, Henninger and Bollag, (1976); 21, Reddy (1964).

N_2O, NO, N_2 and O_2 by the GC-ultrasonic method described by Blackmer and Bremner (1977). The analyses for O_2 were performed to check that anaerobic conditions had been maintained. The results of analyses for NO are not reported because only trace amounts of this gas could be detected. All experiments were performed in triplicate.

Aqueous solutions of the nitrification inhibitors were prepared immediately before use. Test compounds with low water solubility were emulsified into water with a high speed mixer (Omni-Mixer supplied by Ivan Sorval, Inc., Norwalk, CN).

Analyses of soil samples for nitrite were performed by the colorimetric procedure described by Bremner (1965). Analyses for nitrate were performed by the steam distillation method described by Bremner and Keeney (1966).

No loss of nitrate or production of nitrite, N_2O, NO or N_2 could be detected when soil samples previously sterilized by autoclaving at 121°C for 1 hour were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3.

RESULTS AND DISCUSSION

Table 8 shows that only one of the nitrification inhibitors studied (potassium azide) retarded denitrification of nitrate when applied at the rate of 10 μg g^{-1} soil, and Table 9 shows that only two (potassium azide and 2,4-diamino-6-trichloromethyl-*s*-triazine) inhibited denitrification when applied at the rate of 50 μg g^{-1} soil. The other nitrification inhibitors studied either had no appreciable effect on denitrification, or enhanced denitrification, when applied at the rate of 10 or 50 μg g^{-1} soil, enhancement being most marked with 3-mercapto-1,2,4-triazole. It is noteworthy that 3-mercapto-1,2,4-triazole and phenylmercuric acetate promoted accumulation of NO_2^--N when applied at the rate of 50 μg g^{-1} soil (Table 3).

Table 9 shows that, when applied at the rate of 50 μg g^{-1} soil, several of the inhibitors studied decreased the ratio of N_2/N_2O in the gaseous products of denitrification (e.g., N-Serve 24E, potassium azide, 3-mercapto-1,2,4-triazole, sulfathiazole, sodium ethylxanthate, thiourea and dicyandiamide), but that others increased this ratio (e.g., potassium ethylxanthate, sodium isopropylxanthate, sodium thiocarbonate, sodium diethyldithiocarbamate and phenylmercuric acetate).

Table 10 shows the data obtained when the experiments reported in Table 9 were repeated using soil samples that had been amended with available organic carbon (as mannitol) to promote denitrification of nitrate by soil microorganisms. The effect of adding available organic carbon was investigated because in several previous studies of the

Table 8. Effects of 10 μg g^{-1} soil of various nitrification inhibitors on denitrification of nitrate in soil[a]

Nitrification inhibitor	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	μg g^{-1} soil				
None	109	0	34	74	108
Nitrapyrin (N-Serve)	109	0	36	72	108
Nitrapyrin (N-Serve 24)	110	0	34	75	109
Nitrapyrin (N-Serve 24E)	111	0	39	72	111
Potassium azide	88	0	1	87	88
2-Amino-4-chloro-6-methylpyrimidine	108	0	32	75	107
2-Mercaptobenzothiazole	110	0	38	72	110
Sulfathiazole	108	0	39	68	107
Etridiazole (Dwell)	109	0	33	75	108
4-Amino-1,2,4-triazole	109	0	39	70	109
3-Mercapto-1,2,4-triazole	126	0	58	70	128
2,4-Diamino-6-trichloromethyl-*s*-triazine	108	0	31	76	107
Potassium amylxanthate	112	0	38	74	112
Potassium ethylxanthate	107	0	26	81	107
Sodium ethylxanthate	113	0	40	73	113
Sodium isopropylxanthate	110	0	30	79	109
Guanylthiourea	109	0	34	75	109
Thiourea	109	0	35	74	109
2-Chloroacetamide	110	0	35	74	109
2-Fluoroacetamide	107	0	35	72	107
4-Nitrobenzotrichloride	107	0	32	75	109
4-Mesylbenzotrichloride	111	0	34	76	110
Caffeic acid	109	0	33	74	107
Chlorogenic acid	110	0	34	77	111
p-Coumaric acid	107	0	33	75	108
Ferulic acid	109	0	35	74	109
Gallic acid	108	0	35	73	108
Sodium thiocarbonate	111	0	30	81	111
Sodium diethyldithiocarbamate	110	0	38	73	111
Phenylmercuric acetate	116	19	20	78	117
Dicyandiamide	108	0	39	69	108

[a] 30-g samples of Canisteo soil were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 0.3 mg of the inhibitor specified.

Table 9. Effects of 50 μg g^{-1} soil of various nitrification inhibitors on denitrification of nitrate in soil[a]

Nitrification inhibitor	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	μg g^{-1} soil				
None	109	0	34	74	108
Nitrapyrin (N-Serve)	114	0	38	75	114
Nitrapyrin (N-Serve 24)	118	0	42	76	118
Nitrapyrin (N-Serve 24E)	116	0	47	69	116
Potassium azide	43	0	27	16	43
2-Amino-4-chloro-6-methylpyrimidine	107	0	43	66	109
2-Mercaptobenzothiazole	114	0	44	69	113
Sulfathiazole	107	0	43	65	108
Etridiazole (Dwell)	115	0	38	78	116
4-Amino-1,2,4-triazole	109	0	44	67	111
3-Mercapto-1,2,4-triazole	160	58	60	42	160
2,4-Diamino-6-trichloromethyl-*s*-triazine	97	0	26	71	97
Potassium amylxanthate	128	0	53	77	130
Potassium ethylxanthate	110	0	13	97	110
Sodium ethylxanthate	128	0	59	70	129
Sodium isopropylxanthate	109	0	8	103	111
Guanylthiourea	111	0	36	76	112
Thiourea	108	0	57	50	107
2-Chloroacetamide	120	0	41	78	119
2-Fluoroacetamide	107	0	45	62	107
4-Nitrobenzotrichloride	109	0	33	74	107
4-Mesylbenzotrichloride	108	0	35	75	110
Caffeic acid	109	0	34	74	108
Chlorogenic acid	112	0	36	75	111
p-Coumaric acid	125	0	34	92	126
Ferulic acid	108	0	36	72	108
Gallic acid	109	0	34	76	110
Sodium thiocarbonate	110	0	22	89	111
Sodium diethyldithiocarbamate	123	0	12	111	123
Phenylmercuric acetate	127	36	15	75	126
Dicyandiamide	109	0	44	66	110

[a] 30-g samples of Canisteo soil were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 1.5 mg of the inhibitor specified.

effects of nitrification inhibitors on denitrification, the inhibitors were added to soil in an organic solvent (ethanol or methanol) that is readily utilized by soil microorganisms (Henninger and Bollag, 1976; Mills, 1984) or were added to soil amended with available organic carbon (e.g., glucose) (Mills et al., 1976; Mills and Pokorny, 1978; Cribbs and Mills, 1979; McElhannon and Mills, 1981a). Comparison of the data in Tables 9 and 10 shows that six of the inhibitors which did not retard denitrification when applied at the rate of 50 $\mu g\ g^{-1}$ soil to soil that was not amended with available organic carbon did inhibit denitrification when applied at this rate to soil amended with mannitol (sulfathiazole, potassium ethylxanthate, sodium isopropylxanthate, 4-nitrobenzotrichloride, sodium thiocarbonate and phenylmercuric acetate). Previous work by Henninger and Bollag (1976) showed that potassium azide, sulfathiazole and phenylmercuric acetate inhibited denitrification in soil when they were dissolved in ethanol and applied at rates as low as 5 $\mu g\ g^{-1}$ soil.

Table 11 shows the effects of different amounts of nitrapyrin (as N-Serve, N-Serve 24 and N-Serve 24E), etridiazole (Dwell) and potassium azide on denitrification of nitrate in Canisteo and Harps soil. Comparison of the data in Tables 8, 9, and 11 shows that, whereas potassium azide inhibited denitrification in both soils when applied at rates as low as 10 $\mu g\ g^{-1}$ soil, N-Serve, N-Serve 24, N-Serve 24E and Dwell had no inhibitory effect on denitrification when applied at the rate of 10 $\mu g\ g^{-1}$ soil and enhanced denitrification when applied at the rate of 50 or 100 $\mu g\ g^{-1}$ soil. It is noteworthy that N-Serve 24 and

Table 10. Effects of 50 μg g^{-1} soil of various nitrification inhibitors on denitrification of nitrate in soil amended with mannitol[a]

Nitrification inhibitor	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	---------- μg g^{-1} soil ----------				
None	278	203	66	9	278
Nitrapyrin (N-Serve)	301	221	56	24	301
Nitrapyrin (N-Serve 24)	296	247	41	8	296
Nitrapyrin (N-Serve 24E)	287	255	22	11	288
Potassium azide	9	0	2	6	8
2-Amino-4-chloro-6-methylpyrimidine	278	206	63	9	278
2-Mercaptobenzothiazole	289	236	44	10	290
Sulfathiazole	155	118	28	9	155
Etridiazole (Dwell)	264	228	27	9	264
4-Amino-1,2,4-triazole	262	198	55	9	262
3-Mercapto-1,2,4-triazole	277	261	13	5	279
2,4-Diamino-6-trichloromethyl-*s*-triazine	275	208	56	11	275
Potassium amylxanthate	303	241	56	6	303
Potassium ethylxanthate	105	92	7	6	105
Sodium ethylxanthate	267	236	26	7	269
Sodium isopropylxanthate	199	174	14	11	199
Guanylthiourea	281	208	64	9	281
Thiourea	255	195	54	6	255
2-Chloroacetamide	270	215	48	8	271
2-Fluoroacetamide	280	222	44	14	280
4-Nitrobenzotrichloride	188	153	26	11	190
4-Mesylbenzotrichloride	303	219	72	10	301
Caffeic acid	270	198	64	9	271
Chlorogenic acid	271	197	65	9	271
p-Coumaric acid	266	200	57	9	266
Ferulic acid	262	197	56	11	264
Gallic acid	268	198	63	7	268
Sodium thiocarbonate	204	182	12	11	205
Sodium diethyldithiocarbamate	275	198	68	10	276
Phenylmercuric acetate	129	109	8	12	129
Dicyandiamide	290	215	64	11	290

[a] 30-g samples of Canisteo soil were incubated (30°C, 15 mL of H_2O) under He for 1 day after treatment with 9 mg of nitrate-N as KNO_3, 4.5 mg of carbon as mannitol and 1.5 mg of the inhibitor specified.

Table 11. Effects of different amounts of various nitrification inhibitors on denitrification of nitrate in soil[a]

Nitrification inhibitor	Amount added	NO_3^--N lost	N produced			
			NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	----------	μg g^{-1} soil	----------			
		Harps Soil				
None		145	0	22	123	145
Nitrapyrin	10	145	0	7	137	144
(N-Serve)	50	152	0	13	138	151
	100	156	0	15	142	157
Nitrapyrin	10	146	0	20	126	146
(N-Serve 24)	50	154	0	41	114	155
	100	162	0	72	90	162
Nitrapyrin	10	144	0	20	125	145
(N-Serve 24E)	50	154	0	46	107	153
	100	165	0	76	89	165
Etridiazole	10	145	0	20	124	144
(Dwell)	50	150	0	23	128	151
	100	153	0	24	129	153
Potassium azide	10	110	0	0	110	110
	25	103	0	46	56	102
	50	56	0	37	18	55
		Canisteo Soil				
None		109	0	34	74	108
Nitrapyrin (N-Serve)	100	116	0	65	51	116
Nitrapyrin (N-Serve 24)	100	130	15	63	53	131
Nitrapyrin (N-Serve 24E)	100	133	16	53	64	133
Etridiazole (Dwell)	100	115	0	37	79	116
Potassium azide	25	63	0	31	32	63

[a] 30-g samples of soil were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and the amount of inhibitor specified.

N-Serve 24E promoted accumulation of N_2O in both soils when applied at the rate of 50 or 100 μg g^{-1} soil and promoted NO_2^- accumulation in the Canisteo soil when applied at the rate of 100 μg g^{-1} soil.

My finding that nitrapyrin and etridiazole had no effect on denitrification when applied at the rate of 10 μg g^{-1} soil and enhanced denitrification when applied at the rate of 50 or 100 μg g^{-1} soil is contrary to findings by Mitsui et al. (1964), McElhannon and Mills (1981a; 1981b), Mills and McElhannon (1983), Mills (1984) and Mills and McElhannon (1984) because they reported that small amounts (0.5-14 μg g^{-1} soil) of nitrapyrin or etridiazole inhibited denitrification in soil. However, the analyses performed by these workers did not permit reliable conclusions concerning the effects of nitrapyrin and etridiazole on denitrification in soil (they did not, as in work presented here, demonstrate that loss of nitrate-N by denitrification could be accounted for by production of N as nitrite, N_2O and N_2). This criticism also applies to reports that nitrapyrin inhibited denitrification in sand-bark media (Mills and Pokorny, 1978) or in a humic peat (Cribbs and Mills, 1979) when applied at the rate of 50 μg g^{-1} organic medium.

To summarize, only one of the nitrification inhibitors studied (potassium azide) inhibited denitrification when applied at the rate of 10 μg g^{-1} soil, and only two (potassium azide and 2,4-diamino-6-trichloromethyl-*s*-triazine) inhibited denitrification when applied at the rate of 50 μg g^{-1} soil. The other inhibitors either had no appreciable effect on denitrification, or enhanced denitrification, when applied at

these rates. Reports that denitrification in soil is inhibited by small amounts of nitrapyrin and etridiazole could not be confirmed.

SUMMARY

The influence of 28 nitrification inhibitors on denitrification of nitrate in soil was studied by determining the effects of different amounts of each inhibitor on the amounts of nitrate lost and the amounts of nitrite, N_2O and N_2 produced when soil samples were incubated anaerobically after treatment with nitrate or with nitrate and mannitol. The inhibitors used included nitrapyrin (N-Serve), etridiazole (Dwell), potassium azide, 2-amino-4-chloro-6-methylpyrimidine (AM), sulfathiazole (ST), 4-amino-1,2,4-triazole (ATC), 2,4-diamino-6-trichloromethyl-*s*-triazine (CL-1580), potassium ethylxanthate, guanylthiourea (ASU), 4-nitrobenzotrichloride, 4-mesylbenzotrichloride, sodium thiocarbonate (STC), phenylmercuric acetate (PMA) and dicyandiamide (DCD).

Only one of the nitrification inhibitors studied (potassium azide) retarded denitrification when applied at the rate of 10 $\mu g\ g^{-1}$ soil, and only two (potassium azide and 2,4-diamino-6-trichloromethyl-*s*-triazine) inhibited denitrification when applied at the rate of 50 $\mu g\ g^{-1}$ soil. The other inhibitors either had no appreciable effect on denitrification, or enhanced denitrification, when applied at the rate of 10 or 50 $\mu g\ g^{-1}$ soil, enhancement being most marked with 3-mercapto-1,2,4-triazole. Seven of the inhibitors (potassium azide, sulfathiazole, potassium ethylxanthate, sodium isopropylxanthate, 4-nitrobenzotrichloride, sodium thiocarbonate and phenylmercuric acetate) retarded denitrification when applied at the rate of 50 $\mu g\ g^{-1}$ soil to soil amended with mannitol to promote microbial activity.

Reports that nitrapyrin (N-Serve) and etridiazole (Dwell) inhibit denitrification when applied at rates as low as 0.5 $\mu g\ g^{-1}$ soil could not be confirmed. No inhibition of denitrification was observed when these compounds were applied at the rate of 10 $\mu g\ g^{-1}$ soil, and enhancement of denitrification was observed when they were applied at the rate of 50 or 100 $\mu g\ g^{-1}$ soil.

PART III. EFFECTS OF HERBICIDES ON DENITRIFICATION IN SOIL

INTRODUCTION

Herbicides are now used extensively in crop production. For example, in 1980, 92% of the corn acreage, 93% of the soybean acreage and 17% of the pasture acreage in Iowa received herbicides. The increasing use of herbicides with nitrogen fertilizers has created concern about the potential adverse effect of the pesticides on non-target organisms and the environment, and it has emphasized the need for information concerning the effects of herbicides on nitrogen transformations in soil. Numerous studies of the effects of herbicides on nitrification of ammonium in soil have been reported, but very little is known about the effects of herbicides on denitrification in soil (Goring and Laskowski, 1982).

Denitrifying bacterial populations in soil have been reported to be enhanced by PCP (pentachlorophenol) (Ishizawa and Matsuguchi, 1966) and by 2,4-D (2,4-dichlorophenoxyacetic acid) (Sethunathan, 1970), but to be decreased by MCPA (2-methyl-4-chlorophenoxyacetic acid) and propanil (De and Mukhopadhyay, 1971). Denitrification by cultures of denitrifying bacteria has been reported to be inhibited by diquat (Atkinson, 1973), MCPA, 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) (Torstensson, 1974), 2,4-D, chlorbromuron, chloroxuron, fluometuron, metoxuron, neburon, diuron, linuron, siduron (Hart and Larson, 1966; Bollag and Nash, 1974; Bollag and Henninger, 1976), dalapon (Grant and Payne, 1982), simazine and atrazine (McElhannon et al., 1984; Mills, 1984), to be enhanced by MCPA, 2,4,5-T, linuron and simazine (Torstensson, 1974), and to be

unaffected by diquat (Atkinson, 1973), atrazine and simazine (Bollag and Henninger, 1976). Other herbicides found to have no effect on denitrification by cultures of denitrifying bacteria include fenuron, metobromuron, monuron, ametryne, endothal, hydroxysimazine and propham (Bollag and Nash, 1974; Bollag and Henninger, 1976).

PCP (Mitsui et al., 1964) and metolachlor (Bollag and Kurek, 1980) have been found to inhibit denitrification in soil. Eight herbicides reported to inhibit denitrification by cultures of denitrifying bacteria have also been reported to inhibit denitrification in soil. These are 2,4-D (Bollag and Henninger, 1976), fluometuron (Bollag and Kurek, 1980), dalapon (Weeraratna, 1980), diuron, linuron, neburon, simazine and atrazine (Rolston and Cervelli, 1980; McElhannon et al., 1984; Mills, 1984). Atrazine has also been reported to enhance denitrification in soil (Cervelli and Rolston, 1983) and to have no effect on denitrification in soil (Henninger and Bollag, 1976). Other herbicides that appear to have no effect on denitrification in soil are PCP (Mitsui et al., 1964), diuron, linuron, simazine, ametryne, endothal, hydroxysimazine, propham and siduron (Bollag and Henninger, 1976).

My purpose was to determine the effects of 20 extensively used herbicides on denitrification of nitrate in soil. The herbicides studied are commonly used for weed control in corn and soybeans (butylate, EPTC, chlorpropham, linuron, alachlor, trifluralin, atrazine, cyanazine, metribuzin, simazine, dalapon, chloramben, dicamba and dinoseb) or for weed control in hay and pasture (propham, diuron, monuron, siduron, 2,4-D amine and 2,4-D ester).

MATERIALS AND METHODS

The soils used (Table 12) were surface (0-15 cm) samples of soils representative of the Webster, Harps and Storden series used extensively for corn and soybean production in central Iowa. The analyses reported in Table 12 were performed as described by Zantua and Bremner (1975). Before use, each soil was air-dried and sieved (<2 mm).

Table 12. Analyses of soils

Soil			Organic	Total		
Series	Subgroup	pH	carbon	nitrogen	Sand	Clay
			---------- % ----------			
Webster	Typic Haplaquoll	7.5	3.3	0.24	31	30
Harps	Typic Calciaquoll	7.7	6.6	0.50	10	41
Storden	Typic Udorthent	8.1	0.5	0.05	13	28

The names, formulations and sources of the 20 herbicides studied are reported in Table 13. To study the effects of these compounds on denitrification of nitrate in soil, 30-g samples of air-dried soil were placed in 1.2-L flasks fitted with standard taper (34/45) ground-glass joints and were treated with 5 mL of H_2O containing 9 mg N as KNO_3 and with 10 mL of H_2O containing 0, 0.3 or 1.5 mg of the herbicide under study. Each flask was then sealed with a glass stopper fitted with a standard taper (34/45) ground-glass joint and a glass stopcock and was connected to a manifold system attached to a mercury (Hg) manometer. The

Table 13. Herbicides studied

Common name	Chemical name	Formulation[a]	Source[b]
Butylate	Bis(2-methylpropyl)carbamothioic acid *S*-ethyl ester	EC(800)	S
EPTC	Dipropylcarbamothioic acid *S*-ethyl ester	EC(720)	S
Chlorpropham	(3-Chlorophenyl)carbamic acid 1-methylethyl ester	EC(480)	P
Propham	Phenylcarbamic acid 1-methylethyl ester	EC(240)	P
Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethylurea	WP(80)	PN
Linuron	*N'*-(3,4-Dichlorophenyl)-*N*-methoxy-*N*-methylurea	WP(80)	PN
Monuron	*N'*-(4-Chlorophenyl)-*N*,*N*-dimethylurea	WP(80)	PN
Siduron	*N*-(2-Methylcyclohexyl)-*N'*-phenylurea	WP(80)	PN
Alachlor	2-Chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide	EC(480)	MO
Trifluralin	2,6-Dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine	EC(480)	E
2,4-D amine	(2,4-Dichlorophenoxy)acetic acid, dimethylamine salt	L(480)	A
2,4-D ester	(2,4-Dichlorophenoxy)acetic acid, butoxy ethanol ester	EC(480)	A
Atrazine	6-Chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine	WP(80)	C
Cyanazine	2{[4-Chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino}-2-methylpropanenitrile	WP(80)	SC
Metribuzin	4-Amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one	WP(50)	M
Simazine	6-Chloro-*N*,*N'*-diethyl-1,3,5-triazine-2,4-diamine	WP(80)	C
Dalapon	2,2-Dichloropropanoic acid	WP(85)	D
Chloramben	3-Amino-2,5-dichlorobenzoic acid	L(240)	A
Dicamba	3,6-Dichloro-2-methoxybenzoic acid	L(480)	V
Dinoseb	2-(1-Methylpropyl)-4,6-dinitrophenol	L(360)	D

[a] EC, emulsifiable concentrate; WP, wettable powder; L, liquid. Values in parentheses after EC and L indicate grams of active ingredient per liter of formulation. Values in parentheses after WP indicate percent (w/w) of active ingredient in formulation.

[b] S, Stauffer Chemical Co., Westport CT; P, PPG Industries Inc., Pittsburgh, PA; PN, duPont de Nemours & Co., Wilmington, DE; MO, Monsanto Agricultural Products Co., St. Louis, MO; E, Elanco Products Co., Indianapolis, IN; A, Amchem Products, Inc., Ambler, PA; C, Ciba-Geigy Corp., Greensboro, NC; SC, Shell Chemical Co., Houston, TX; M, Mobay Chemical Corp., St. Louis, MO; D, Dow Chemical U.S.A., Midland, MI; V, Velsicol Chemical Corp., Chicago, IL.

flasks were evacuated via the stopcock and filled with helium (He) to slightly above atmospheric pressure, and this process was repeated three times. The flasks were then brought to atmospheric pressure with He and placed in an incubator at 30°C. The atmospheres in the flasks were subsequently sampled at 2-day intervals for determination of N_2O, NO, N_2 and O_2 by the GC-ultrasonic detector method described by Blackmer and Bremner (1977). Only the data obtained after 8 days are reported because the conclusions reached were not significantly affected by time of sampling. The analyses for O_2 were performed to check that anaerobic conditions had been maintained. The results of analyses for NO are not reported because only trace amounts of this gas could be detected. All experiments were performed in triplicate.

Soil samples were analysed for nitrite by the colorimetric procedure described by Bremner (1965). Nitrate was determined by the steam distillation method described by Bremner and Keeney (1966).

No loss of nitrate or production of nitrite, N_2O, NO or N_2 could be detected when soil samples previously sterilized by autoclaving at 121°C for 1 hour were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3.

RESULTS AND DISCUSSION

None of the herbicides had a significant effect on denitrification of nitrate when applied at the rate of 10 μg g^{-1} soil, but dinoseb increased the ratio of N_2/N_2O in the gaseous products of denitrification (i.e., promoted reduction of N_2O by soil microorganisms) (Tables 14-16).

Butylate, EPTC, diuron, atrazine, simazine and dalapon had no significant effect on denitrification when applied at the rate of 50 μg g^{-1} soil (Tables 17-19). Rolston and Cervelli (1980) found that diuron inhibited denitrification in soil when applied at the rate of 30 μg g^{-1} soil, whereas Bollag and Henninger (1976) found that it had no effect when applied at the rate of 100 μg g^{-1} soil. Bollag and Henninger (1976) also found that atrazine and simazine had no effect on denitrification when applied at the rate of 100 μg g^{-1} soil, and Grant and Payne (1982) found that atrazine at rates up to 1250 μg g^{-1} sediment had no effect on denitrification in a salt marsh sediment. However, atrazine has been reported to enhance denitrification in a soil column when applied at the rate of 3 μg g^{-1} soil (Cervelli and Rolston, 1983). Moreover, atrazine applied at the rate of 28.5 μg g^{-1} soil (Rolston and Cervelli, 1980) and atrazine and simazine applied at rates as low as 0.5 μg g^{-1} soil (McElhannon et al., 1984) have been reported to inhibit denitrification in soil. Dalapon has been reported to inhibit denitrification in soil when applied at the rate of 10 μg g^{-1} soil (Weeraratna, 1980) and to inhibit denitrification in a salt marsh sediment when applied at the rate of 12.5 μg g^{-1} sediment (Grant and

Table 14. Effects of 10 μg g^{-1} soil of various herbicides on denitrification of nitrate in Webster soil[a]

Herbicide	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	μg g^{-1} soil				
None	120	0	30	90	120
Butylate	119	0	29	91	120
EPTC	121	0	30	91	121
Chlorpropham	119	0	30	90	120
Propham	120	0	24	96	120
Diuron	121	0	32	89	121
Linuron	119	0	29	90	119
Monuron	119	0	30	90	120
Siduron	120	0	30	90	120
Alachlor	119	0	26	93	119
Trifluralin	120	0	28	92	120
2,4-D amine	120	0	31	90	121
2,4-D ester	121	0	30	91	121
Atrazine	122	0	30	92	122
Cyanazine	118	0	28	91	119
Metribuzin	122	0	32	91	123
Simazine	120	0	30	90	120
Dalapon	122	0	31	92	123
Chloramben	120	0	30	91	121
Dicamba	118	0	26	93	119
Dinoseb	118	0	0	119	119

[a] 30-g samples of soil were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 0.3 mg of the herbicide specified.

Table 15. Effects of 10 μg g^{-1} soil of various herbicides on denitrification of nitrate in Harps soil[a]

Herbicide	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	μg g^{-1} soil				
None	142	0	35	107	142
Butylate	143	0	34	108	142
EPTC	141	0	36	105	141
Chlorpropham	143	0	32	110	142
Propham	143	0	29	114	143
Diuron	141	0	34	107	141
Linuron	142	0	36	107	143
Monuron	142	0	33	110	143
Siduron	140	0	35	105	140
Alachlor	142	0	36	106	142
Trifluralin	144	0	32	112	144
2,4-D amine	142	0	36	107	143
2,4-D ester	141	0	35	105	140
Atrazine	142	0	35	106	141
Cyanazine	142	0	33	108	141
Metribuzin	142	0	34	108	142
Simazine	140	0	37	104	141
Dalapon	140	0	33	107	140
Chloramben	140	0	35	106	141
Dicamba	143	0	36	107	143
Dinoseb	150	0	0	150	150

[a] 30-g samples of soil were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 0.3 mg of the herbicide specified.

Table 16. Effects of 10 μg g^{-1} soil of various herbicides on denitrification of nitrate in Storden soil[a]

Herbicide	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	μg g^{-1} soil				
None	57	4	14	39	57
Butylate	57	5	13	39	57
EPTC	56	6	12	40	58
Chlorpropham	57	4	14	37	55
Propham	57	4	14	39	57
Diuron	55	6	14	37	57
Linuron	60	4	16	40	60
Monuron	58	4	13	41	58
Siduron	59	4	15	40	59
Alachlor	57	5	13	38	56
Trifluralin	60	5	14	41	60
2,4-D amine	58	4	16	38	58
2,4-D ester	61	6	15	40	61
Atrazine	55	6	15	36	57
Cyanazine	57	4	13	40	57
Metribuzin	59	5	14	40	59
Simazine	60	5	17	38	60
Dalapon	57	4	13	40	57
Chloramben	57	5	15	37	57
Dicamba	59	5	16	37	58
Dinoseb	60	0	0	61	61

[a] 30-g samples of soil were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 0.3 mg of the herbicide specified.

Table 17. Effects of 50 μg g^{-1} soil of various herbicides on denitrification of nitrate in Webster soil[a]

Herbicide	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	μg g^{-1} soil				
None	120	0	30	90	120
Butylate	119	1	15	103	119
EPTC	120	1	18	101	120
Chlorpropham	128	0	6	121	127
Propham	159	1	22	136	159
Diuron	118	0	36	83	119
Linuron	110	0	32	78	110
Monuron	118	0	40	78	118
Siduron	118	0	28	90	118
Alachlor	129	0	0	129	129
Trifluralin	116	1	35	80	116
2,4-D amine	120	0	19	101	120
2,4-D ester	117	0	22	95	117
Atrazine	122	0	32	90	122
Cyanazine	114	0	36	77	113
Metribuzin	135	0	39	97	136
Simazine	118	0	29	90	119
Dalapon	115	0	30	87	117
Chloramben	119	1	10	108	119
Dicamba	118	0	0	118	118
Dinoseb	146	0	0	146	146

[a] 30-g samples of soil were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 1.5 mg of the herbicide specified.

Table 18. Effects of 50 μg g^{-1} soil of various herbicides on denitrification of nitrate in Harps soil[a]

Herbicide	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	μg g^{-1} soil				
None	142	0	35	107	142
Butylate	149	0	38	112	150
EPTC	142	0	48	94	142
Chlorpropham	150	0	40	110	150
Propham	159	0	10	150	160
Diuron	145	0	32	112	144
Linuron	144	0	31	113	144
Monuron	150	0	30	120	150
Siduron	144	0	20	125	145
Alachlor	152	0	14	138	152
Trifluralin	166	0	39	127	166
2,4-D amine	152	0	20	132	152
2,4-D ester	160	0	16	146	162
Atrazine	148	0	31	117	148
Cyanazine	143	0	28	116	144
Metribuzin	152	0	34	120	154
Simazine	140	0	28	112	140
Dalapon	142	0	32	111	143
Chloramben	157	0	38	120	158
Dicamba	152	1	6	145	152
Dinoseb	172	0	0	172	172

[a] 30-g samples of soil were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 1.5 mg of the herbicide specified.

Table 19. Effects of 50 μg g^{-1} soil of various herbicides on denitrification of nitrate in Storden soil[a]

Herbicide	NO_3^--N lost	N produced			
		NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	μg g^{-1} soil				
None	57	4	14	39	57
Butylate	54	4	6	44	54
EPTC	50	1	2	47	50
Chlorpropham	69	30	3	35	68
Propham	60	13	4	43	60
Diuron	64	14	8	42	64
Linuron	62	19	5	39	63
Monuron	80	19	7	54	80
Siduron	75	2	9	66	77
Alachlor	51	6	3	42	51
Trifluralin	82	14	11	58	83
2,4-D amine	85	9	18	58	85
2,4-D ester	92	10	17	65	92
Atrazine	60	5	5	50	60
Cyanazine	65	4	14	47	65
Metribuzin	70	12	14	45	71
Simazine	61	6	13	42	61
Dalapon	61	6	13	42	61
Chloramben	59	7	10	42	59
Dicamba	64	5	8	50	63
Dinoseb	85	0	0	85	85

[a] 30-g samples of soil were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 1.5 mg of the herbicide specified.

Payne, 1982).

Tables 17-19 show that metribuzin and dinoseb enhanced denitrification in the three soils used when they were applied at the rate of 50 μg g^{-1} soil. They also show that dinoseb increased the ratio of N_2/N_2O in the gaseous products of denitrification in these soils and that metribuzin inhibited nitrite reduction in the Storden soil when applied at the rate of 50 μg g^{-1} soil. Six of the herbicides (chlorpropham, propham, alachlor, 2,4-D amine, 2,4-D ester and dicamba) either had no significant effect on denitrification, or enhanced denitrification, when applied at the rate of 50 μg g^{-1} soil, but increased the ratio of N_2/N_2O in the gaseous products of denitrification (i.e., promoted N_2O reduction by soil microorganisms) when applied at this rate. Chlorpropham and propham inhibited nitrite reduction in the Storden soil (Table 19). 2,4-D has been reported to inhibit denitrification in soil when applied at the rate of 100 μg g^{-1} soil, to inhibit denitrification by a culture of denitrifying bacteria when applied at the rate of 100 μg mL^{-1} culture medium (Bollag and Henninger, 1976) and to inhibit denitrification in a salt marsh sediment when applied at the rate of 12.5 μg g^{-1} sediment (Grant and Payne, 1982). Bollag and Henninger (1976) found that propham had no effect on denitrification in soil when applied at the rate of 100 μg g^{-1} soil or on denitrification by a culture of denitrifying bacteria when applied at the rate of 100 μg mL^{-1} culture medium.

When applied at the rate of 50 μg g^{-1} soil, the effects of six of the herbicides studied (linuron, monuron, siduron, trifluralin, cyanazine

and chloramben) depended on the soil used. Linuron inhibited denitrification in the Webster soil (Table 17), had no effect on denitrification in the Harps soil (Table 18) and inhibited nitrite reduction and N_2O production in the Storden soil (Table 19). Linuron has been reported to inhibit denitrification by a culture of denitrifying bacteria when applied at rates of 50, 100 and 200 $\mu g\ mL^{-1}$ culture medium (Bollag and Nash, 1974) and to inhibit denitrification in soil when applied at the rate of 26.5 $\mu g\ g^{-1}$ soil (Rolston and Cervelli, 1980). In contrast, linuron has been reported to have no effect on denitrification in soil when applied at the rate of 100 $\mu g\ g^{-1}$ soil (Bollag and Henninger, 1976).

Monuron and siduron enhanced denitrification in the Storden soil (Table 19) and increased the ratio of N_2/N_2O in the gaseous products of denitrification in the Harps and Storden soils when applied at the rate of 50 $\mu g\ g^{-1}$ soil (Tables 18 and 19). Monuron also inhibited N_2O reduction in the Webster soil (Table 17) and inhibited nitrite reduction in the Storden soil when applied at this rate (Table 19). Monuron has been reported to have no effect on denitrification by a culture of denitrifying bacteria when applied at rates up to 200 $\mu g\ mL^{-1}$ culture medium (Bollag and Nash, 1974). Siduron has been reported to inhibit denitrification in soil when applied at the rate of 100 $\mu g\ g^{-1}$ soil and to inhibit denitrification by a culture of denitrifying bacteria when applied at the rate of 100 $\mu g\ mL^{-1}$ culture medium (Bollag and Nash, 1974; Bollag and Henninger, 1976).

Trifluralin enhanced denitrification in the Harps and Storden soils

when applied at the rate of 50 μg g^{-1} soil (Tables 18 and 19), but inhibited nitrite reduction in the Storden soil when applied at this rate. Cyanazine inhibited denitrification and caused a decrease in the ratio of N_2/N_2O in the gaseous products of denitrification when applied at the rate of 50 μg g^{-1} soil to the Webster soil (Table 17), but it had no effect on denitrification in the Harps soil (Table 18), and it enhanced denitrification in the Storden soil when applied at this rate (Table 19). Chloramben had no effect on denitrification in the Webster (Table 17) and Storden (Table 19) soils when applied at the rate of 50 μg g^{-1} soil, but it enhanced denitrification in the Harps soil when applied at this rate (Table 18). It also increased the ratio of N_2/N_2O in the gaseous products of denitrification in the Webster soil (i.e., promoted reduction of N_2O by soil microorganisms) (Table 17).

To sumarize, none of the herbicides studied had a significant effect on denitrification when applied at the rate of 10 μg g^{-1} soil. Butylate, EPTC, diuron, simazine and dalapon had no significant effect on denitrification when applied at the rate of 50 μg g^{-1} soil, whereas metribuzin and dinoseb enhanced denitrification when applied at this rate. The influence of the other herbicides on denitrification when applied at the rate of 50 μg g^{-1} soil depended on the soil, but all enhanced or inhibited denitrification in at least one soil.

SUMMARY

The effects of 20 herbicides on denitrification of nitrate in three soils were studied by determining the effects of 10 and 50 μg g^{-1} soil of each herbicide on the amounts of nitrate lost and the amounts of nitrite, N_2O and N_2 produced when soil samples were incubated anaerobically after treatment with nitrate. The herbicides studied were butylate, EPTC, chlorpropham, propham, diuron, linuron, monuron, siduron, alachlor, trifluralin, 2,4-D amine, 2,4-D ester, atrazine, cyanazine, metribuzin, simazine, dalapon, chloramben, dicamba and dinoseb.

None of the herbicides studied significantly affected denitrification of nitrate when applied at the rate of 10 μg g^{-1} soil, but dinoseb increased the ratio of N_2/N_2O in the gaseous products of denitrification when applied at this rate. Butylate, EPTC, diuron, atrazine, simazine and dalapon had no significant effect on denitrification when applied at the rate of 50 μg g^{-1} soil, whereas metribuzin and dinoseb enhanced denitrification when applied at this rate. The influence of the other herbicides on denitrification when applied at the rate of 50 μg g^{-1} soil depended on the soil, but all enhanced or inhibited denitrification in at least one soil.

PART IV. EFFECTS OF INSECTICIDES AND FUNGICIDES ON DENITRIFICATION IN SOIL

INTRODUCTION

The increased use of pesticides with nitrogen fertilizers has emphasized the need for information concerning the effects of pesticides on transformations of fertilizer nitrogen in soil. Numerous studies of the effects of pesticides on nitrification of ammonium in soil have been reported, but very little is known about the effects of pesticides on denitrification in soil (Goring and Laskowski, 1982).

Twelve insecticides [gamma BHC (gamma benzene hexachloride, lindane), carbaryl, carbofuran, DDT (dichlorodiphenyltrichloroethane), diazinon, endrin, methoxychlor, chlordimeform, malathion, temephos, chloropicrin and cartap hydrochloride] have been examined for their effects on denitrification. Gamma BHC applied at the rate of 40 $\mu g\ g^{-1}$ soil (Mitsui et al., 1964), and carbaryl, carbofuran, DDT, diazinon, endrin, methoxychlor (Bollag and Henninger, 1976) and chlordimeform (Bollag and Kurek, 1980) applied at the rate of 100 $\mu g\ g^{-1}$ soil, have been reported to have no effect on denitrification in soil, but carbaryl, malathion and temephos have been reported to inhibit denitrification in a salt marsh sediment when applied at the rate of 1250 $\mu g\ g^{-1}$ sediment (Grant and Payne, 1982). Ishizawa and Matsuguchi (1966) found that chloropicrin increased the population of denitrifying bacteria in soil when applied at the rate of 5 $\mu g\ g^{-1}$ soil, and Endo et al. (1982) found that cartap hydrochloride had no effect on this population when applied at rates up to 100 $\mu g\ g^{-1}$ soil. Bollag and Henninger (1976) found that carbofuran, DDT, diazinon, endrin and

methoxychlor had no effect on denitrification by a pure culture of a denitrifying microorganism when applied at the rate of 100 μg mL^{-1} culture medium, but carbaryl slightly inhibited denitrification by the same microorganism when applied at this rate.

Eleven fungicides [folpet, captafol, DIECA (sodium diethyldithiocarbamate), ziram, dithane, thiram, ferbam, maneb, nabam, captan and terrazole] have been tested for their effects on denitrification. Folpet and captafol applied at the rate of 10 μg g^{-1} soil (Atlas et al., 1978), and DIECA applied at the rate of 100 μg g^{-1} soil (Mitsui et al., 1964), have been reported to have no effect on denitrification in soil, but ziram, dithane, thiram, ferbam, maneb, nabam and captan have been reported to inhibit denitrification in soil when applied at the rate of 50 or 100 μg g^{-1} soil (Mitsui et al., 1964; Bollag and Henninger, 1976). DIECA applied at the rate of 20 μg mL^{-1} culture medium (Matsubara and Mori, 1968), and maneb, nabam and captan applied at the rate of 50 μg mL^{-1} culture medium (Bollag and Henninger, 1976), have been reported to inhibit denitrification by denitrifying bacteria. Mills and McElhannon (1984) reported that terrazole inhibited denitrification in soil when applied at rates as low as 0.5 μg g^{-1} soil and inhibited denitrification in a liquid medium inoculated with soil bacteria when applied at rates as low as 0.02 μg mL^{-1} medium.

The purpose of the research reported here was to determine the effects of several extensively used insecticides and fungicides on denitrification of nitrate in soil. The insecticides studied (lindane, fenitrothion, fonofos, malathion, phorate, terbufos and carbofuran) are

commonly used for control of a variety of insects in a wide range of crops. The fungicides studied (mancozeb, maneb, thiram, benomyl, captan and terrazole) are commonly used for a wide spectrum of plant diseases.

MATERIALS AND METHODS

The soils used (Table 20) were surface (0-15 cm) samples of soils representative of the Webster, Harps and Storden series used extensively for corn and soybean production in central Iowa. The analyses reported in Table 20 were performed as described by Zantua and Bremner (1975). Before use, each soil was air-dried and sieved (<2 mm).

Table 20. Analyses of soils

Soil			Organic	Total		
Series	Subgroup	pH	carbon	nitrogen	Sand	Clay
			%			
Webster	Typic Haplaquoll	7.5	3.3	0.24	31	30
Harps	Typic Calciaquoll	7.7	6.6	0.50	10	41
Storden	Typic Udorthent	8.1	0.5	0.05	13	28

The names, formulations and sources of the seven insecticides and six fungicides studied are reported in Table 21. To determine the effects of these compounds on denitrification of nitrate in soil, 30-g samples of air-dried soil were placed in 1.2-L flasks fitted with standard taper (34/45) ground-glass joints and were treated with 5 mL of H_2O containing 9 mg N as KNO_3 and with 10 mL of H_2O containing 0, 0.3 or 1.5 mg of the compound under study. Each flask was then sealed with a glass stopper fitted with a standard taper (34/45) ground-glass joint and a glass stopcock and was connected to a manifold system attached to a

Table 21. Insecticides and fungicides studied

Common name	Chemical name	Formulation[a]	Source[b]
	Insecticides		
Lindane	1,2,3,4,5,6-Hexachlorocyclohexane	EC(190)	C
Fenitrothion	Phosphorothioic acid *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) ester	EC(960)	S
Fonofos	Ethylphosphonodithioic acid *O*-ethyl *S*-phenyl ester	G(20)	S
Malathion	[(Dimethoxyphosphinothioyl)thio]butanedioic acid diethyl ester	EC(530)	C
Phorate	Phosphorodithioic acid *O,O*-diethyl *S*-[(ethylthio)methyl] ester	G(15)	A
Terbufos	Phosphorodithioic acid *S*-{[(1,1'-dimethylethyl)thio]methyl} *O,O*-diethyl ester	G(15)	A
Carbofuran	2,3-Dihydro-2,2-dimethyl-7-benzofuranol methylcarbamate	G(10)	F
	Fungicides		
Mancozeb	Coordination product of zinc ion and maneb	WP(80)	R
Maneb	{[1,2-Ethanediylbis(carbamodithioato)](2-)}manganese	WP(80)	P
Thiram	Tetramethylthioperoxydicarbonic diamide	WP(65)	P
Benomyl	{1-[(Butylamino)carbonyl]-1H-benzimidazol-2-yl}carbamic acid methyl ester	WP(50)	P
Captan	3a,4,7,7a-Tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1,3(2H)-dione	WP(50)	S
Terrazole	5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole	L(95)	O

[a] EC, emulsifiable concentrate; G, granule; WP, wettable powder; L, liquid. Values in parentheses after EC indicate grams of active ingredient per liter of formulation. Values in parentheses after G and WP indicate percent (w/w) of active ingredient of formulation. Value in parentheses after L indicates percent purity.

[b] C, Chevron Chemical Co., San Francisco, CA; S, Stauffer Chemical Co., Westport, CT; A, American Cyanamid Co., Princeton, NH; F, FMC Corp., Middleport, NY; R, Rohm and Haas Co., Philadelphia, PA; P, duPont de Nemours & Co., Wilmington, DE; O, Olin Corp., Little Rock, AR.

mercury (Hg) manometer. The flasks were evacuated via the stopcock and filled with helium (He) to slightly above atmospheric pressure, and this process was repeated three times. The flasks were then brought to atmospheric pressure with He and placed in an incubator at 30°C. The atmospheres in the flasks were subsequently sampled at 2-day intervals for determination of N_2O, NO, N_2 and O_2 by the GC-ultrasonic detector method described by Blackmer and Bremner (1977). Only the data obtained after 8 days are reported because the conclusions reached were not significantly affected by time of sampling. The analyses for O_2 were performed to check that anaerobic conditions had been maintained. The results of analyses for NO are not reported because only trace amounts of this gas could be detected. All experiments were performed in triplicate.

Soil samples were analysed for nitrite by the colorimetric procedure described by Bremner (1965). Nitrate was determined by the steam distillation method described by Bremner and Keeney (1966).

No loss of nitrate or production of nitrite, N_2O, NO or N_2 could be detected when soil samples previously sterilized by autoclaving at 121°C for 1 hour were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3.

RESULTS AND DISCUSSION

Tables 22-24 show that none of the insecticides studied had a significant effect on denitrification of nitrate in the three soils used when applied at the rate of 10 μg g^{-1} soil, but that most enhanced denitrification when applied at the rate of 50 μg g^{-1} soil. Lindane retarded nitrite reduction in the Harps and Storden soils when applied at the rate of 50 μg g^{-1} soil (Tables 23 and 24), and fenitrothion, phorate, terbufos and carbofuran increased the ratio of N_2/N_2O in the gaseous products of denitrification when applied at this rate (Tables 22-24). In previous work, Mitsui et al. (1964) found that lindane had no effect on denitrification when applied at the rate of 40 μg g^{-1} soil, and Bollag and Henninger (1976) found that carbofuran had no effect on denitrification when applied at the rate of 100 μg g^{-1} soil. Grant and Payne (1982) found that malathion inhibited denitrification when applied at the rate of 1250 μg g^{-1} sediment to a salt marsh sediment.

None of the fungicides studied significantly affected denitrification of nitrate when applied at the rate of 10 μg g^{-1} soil to the three soils used, but thiram increased the ratio of N_2/N_2O in the gaseous products of denitrification (i.e., promoted reduction of N_2O by soil microorganisms) (Tables 22-24). Captan also promoted reduction of N_2O to N_2 in the Harps soil when applied at the rate of 10 μg g^{-1} soil (Table 23), but it retarded N_2O reduction in the Storden soil when applied at this rate (Table 24). Maneb inhibited nitrite reduction in the Storden soil when applied at the rate of 10 μg g^{-1} soil (Table 24).

Table 22. Effects of different amounts of various insecticides and fungicides on denitrification of nitrate in Webster soil[a]

Insecticide or fungicide	Amount added	NO_3^--N lost	N produced			
			NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	----------		μg g^{-1} soil			----------
None		120	0	30	90	120
			Insecticides			
Lindane	10	121	0	29	92	121
	50	132	0	29	102	131
Fenitrothion	10	120	0	23	96	119
	50	116	0	0	118	118
Fonofos	10	125	0	31	94	125
	50	134	0	26	108	134
Malathion	10	128	0	34	94	128
	50	149	0	40	110	150
Phorate	10	122	0	29	93	122
	50	129	0	19	110	129
Terbufos	10	121	0	30	91	121
	50	128	0	24	104	128
Carbofuran	10	119	0	28	92	120
	50	117	0	23	93	116
			Fungicides			
Mancozeb	10	119	0	32	88	120
	50	128	44	50	35	129
Maneb	10	122	0	28	93	121
	50	121	41	11	69	121
Thiram	10	120	0	0	119	119
	50	135	35	3	96	134
Benomyl	10	120	0	33	88	121
	50	111	0	39	72	111
Captan	10	121	0	32	90	122
	50	90	0	21	69	90
Terrazole	10	120	0	29	90	119
	50	121	0	28	93	121

[a] 30-g samples of soil were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 0, 0.3 or 1.5 mg of the insecticide or fungicide specified.

Table 23. Effects of different amounts of various insecticides and fungicides on denitrification of nitrate in Harps soil[a]

Insecticide or fungicide	Amount added	NO_3^--N lost	N produced			
			NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	----------		μg g^{-1} soil			----------
None		142	0	35	107	142
			Insecticides			
Lindane	10	140	0	29	111	140
	50	163	6	38	118	162
Fenitrothion	10	144	0	37	107	144
	50	167	0	0	168	168
Fonofos	10	144	0	31	112	143
	50	150	0	24	126	150
Malathion	10	146	0	40	106	146
	50	164	0	34	130	164
Phorate	10	150	0	34	116	150
	50	171	1	16	156	173
Terbufos	10	143	0	37	106	143
	50	159	0	23	136	159
Carbofuran	10	142	0	34	109	143
	50	148	0	26	123	149
			Fungicides			
Mancozeb	10	140	0	32	108	140
	50	144	18	8	118	144
Maneb	10	142	0	31	112	143
	50	141	4	18	120	142
Thiram	10	146	0	15	130	145
	50	144	1	0	144	145
Benomyl	10	140	0	33	108	141
	50	156	0	34	124	158
Captan	10	144	0	22	124	146
	50	88	0	39	49	88
Terrazole	10	142	0	34	109	143
	50	152	0	12	140	152

[a] 30-g samples of soil were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 0, 0.3 or 1.5 mg of the insecticide or fungicide specified.

Table 24. Effects of different amounts of various insecticides and fungicides on denitrification of nitrate in Storden soil[a]

Insecticide or fungicide	Amount added	NO_3^--N lost	N produced			
			NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
	----------	----------	μg g^{-1} soil	----------	----------	----------
None		57	4	14	39	57
			Insecticides			
Lindane	10	56	6	17	34	57
	50	68	28	2	38	68
Fenitrothion	10	56	4	14	38	56
	50	55	5	1	50	56
Fonofos	10	60	5	12	43	60
	50	69	10	16	43	69
Malathion	10	59	4	15	39	58
	50	85	0	23	62	85
Phorate	10	58	3	14	40	57
	50	56	1	2	53	56
Terbufos	10	57	4	16	37	57
	50	56	0	0	56	56
Carbofuran	10	55	6	14	36	56
	50	65	8	11	46	65
			Fungicides			
Mancozeb	10	56	3	14	40	57
	50	67	28	1	39	68
Maneb	10	60	15	11	34	60
	50	62	39	1	22	62
Thiram	10	58	4	5	49	58
	50	83	56	0	28	84
Benomyl	10	61	6	13	41	60
	50	87	17	23	47	87
Captan	10	55	6	20	29	55
	50	75	24	7	44	75
Terrazole	10	58	3	14	40	57
	50	64	26	3	35	64

[a] 30-g samples of soil were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3 and 0, 0.3 or 1.5 mg of the insecticide or fungicide specified.

Most of the fungicides studied enhanced denitrification of nitrate when applied at the rate of 50 μg g^{-1} soil (Tables 22-24). With all three soils used, maneb and thiram increased the ratio of N_2/N_2O in the gaseous products of denitrification when applied at this rate (i.e., they promoted reduction of N_2O by soil microorganisms), as did mancozeb and terrazole with the Harps and Storden soils, benomyl with the Harps soil and captan with the Storden soil (Tables 22-24). Our finding that maneb, thiram and terrazole had no effect on denitrification when applied at the rate of 10 μg g^{-1} soil and either had no effect on denitrification, or enhanced denitrification when applied at the rate of 50 μg g^{-1} soil (Tables 22-24) is contrary to findings in previous work by Mitsui et al. (1964) and Mills and McElhannon (1984). Mitsui et al. (1964) reported that both maneb and thiram inhibited denitrification when applied at the rate of 50 or 100 μg g^{-1} soil, and Mills and McElhannon (1984) reported that terrazole inhibited denitrification when applied at rates as low as 0.5 μg g^{-1} soil.

Captan inhibited denitrification in the Webster soil (Table 22) and the Harps soil (Table 23) when applied at the rate of 50 μg g^{-1} soil, but it enhanced denitrification in the Storden soil (Table 24) when applied at this rate. In previous work, Bollag and Henninger (1976) found that captan inhibited denitrification of nitrate when applied at the rate of 100 μg g^{-1} soil.

All six of the fungicides studied inhibited nitrite reduction in at least one of the soils used when applied at the rate of 50 μg g^{-1} soil, and three of the fungicides (mancozeb, benomyl and captan) decreased the

ratio of N_2/N_2O in the gaseous products of denitrification in at least one of the soils used when applied at this rate (i.e., they inhibited N_2O reduction by soil microorganisms) (Tables 22-24).

To summarize, none of the insecticides or fungicides studied had a significant effect on denitrification when applied at the rate of 10 $\mu g\ g^{-1}$ soil, and only captan inhibited denitrification when applied at the rate of 50 $\mu g\ g^{-1}$ soil. Reports that maneb, thiram and terrazole inhibit denitrification in soil were not confirmed.

SUMMARY

The effects of seven insecticides and six fungicides on denitrification of nitrate in soils were studied by determining the effects of 10 and 50 $\mu g\ g^{-1}$ soil of each pesticide on the amounts of nitrate lost and the amounts of nitrite, N_2O and N_2 produced when soil samples were incubated anaerobically after treatment with nitrate. The insecticides used were lindane, fenitrothion, fonofos, malathion, phorate, terbufos and carbofuran. The fungicides used were mancozeb, maneb, thiram, benomyl, captan and terrazole.

None of the insecticides studied had a significant effect on denitrification when applied at the rate of 10 $\mu g\ g^{-1}$ soil. When applied at the rate of 50 $\mu g\ g^{-1}$ soil, lindane, fonofos and malathion enhanced denitrification in the three soils studied, whereas fenitrothion, phorate, terbufos and carbofuran either had no appreciable effect on denitrification in these soils, or enhanced denitrification in at least one of the soils.

None of the fungicides studied had an appreciable effect on denitrification when applied at the rate of 10 $\mu g\ g^{-1}$ soil, but thiram increased the ratio of N_2/N_2O in the gaseous products of denitrification. Captan inhibited denitrification in two of three soils studied when applied at the rate of 50 $\mu g\ g^{-1}$ soil. The other five fungicides either had no significant effect on denitrification, or enhanced denitrification, when applied at this rate. Reports that maneb, thiram and terrazole inhibit denitrification in soil were not confirmed.

PART V. EFFECTS OF DALAPON, ATRAZINE AND SIMAZINE ON DENITRIFICATION IN SOIL

INTRODUCTION

In the work reported in Part III, I studied the effects of 20 commonly used herbicides on denitrification of nitrate in soil and found that none of these herbicides significantly affected denitrification when applied at the rate of 10 $\mu g\ g^{-1}$ soil and that most had no effect on denitrification when applied at the rate of 50 $\mu g\ g^{-1}$ soil. The herbicides studied in my work included dalapon (2,2-dichloropropanoic acid), atrazine (6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine) and simazine (6-chloro-*N*,*N'*-diethyl-1,3,5-triazine-2,4-diamine), and my finding that these corn herbicides had no significant effect on denitrification even when applied at the rate of 50 $\mu g\ g^{-1}$ soil was contrary to findings in previous studies of the effects of these herbicides on denitrification (Rolston and Cervelli, 1980; Weeraratna, 1980; Grant and Payne, 1982; McElhannon et al., 1984; Mills, 1984).

Dalapon has been reported to inhibit denitrification in a flooded soil when applied at the rate of 10 $\mu g\ g^{-1}$ soil (Weeraratna, 1980). It also has been reported to inhibit denitrification in a salt marsh sediment when applied at the rate of 12.5 $\mu g\ g^{-1}$ sediment and to retard denitrification by a culture of denitrifying bacteria when applied at the rate of 250 $\mu g\ mL^{-1}$ medium (Grant and Payne, 1982). McElhannon et al. (1984) and Mills (1984) reported that atrazine and simazine inhibited denitrification in a liquid medium inoculated with soil when they were applied at the rate of 2.0 $\mu g\ mL^{-1}$ medium and that these herbicides also

inhibited denitrification in soil when applied at the rate of 0.5 μg g^{-1} soil. Rolston and Cervelli (1980) reported that atrazine inhibited denitrification in soil when applied at the rate of 28.5 μg g^{-1} soil.

The purpose of the work reported here was to try to account for the divergence between my findings and those of previous investigators of the effects of dalapon, atrazine and simazine on denitrification in soil. In my previous work, I studied the effects of 10 and 50 μg g^{-1} soil of these herbicides on denitrification of nitrate in soils that were air-dried but were not amended with available organic carbon before use, denitrification being studied by determining the amounts of nitrate lost and the amounts of nitrite, N_2O and N_2 produced when these soils were incubated anaerobically with nitrate for 8 days. In the work reported here, I performed the same analyses to determine the effects of 5, 10, 25 and 100 μg g^{-1} soil of each herbicide on denitrification of nitrate in soils that were air-dried or kept field-moist before use, and I studied the influence of incubation time and addition of available organic carbon (as glucose) on these effects. The rates of herbicides applied were slightly above or much greater than the recommended rates (see Beste, 1983). Dalapon is used extensively for control of annual and perennial deep-rooted grasses in corn, and it is normally applied at the rate of 10-15 kg ha^{-1} (5-7.5 μg g^{-1} soil). Atrazine is used for long season weed control in corn, and it is normally applied at the rate of 2.2-4.5 kg ha^{-1} (1.1-2.2 μg g^{-1} soil). Simazine is a selective preemergence herbicide used for control of most annual grasses and

broadleaf weeds in corn, and it is normally applied at the rate of 2.2-4.4 kg ha^{-1} (1.1-2.2 μg g^{-1} soil).

MATERIALS AND METHODS

The soils used (Table 25) were surface (0-15 cm) samples of soils representative of the Canisteo, Harps and Storden series used for corn production in central Iowa. After collection, each soil sample was sieved (<2 mm) in the field-moist condition and subsamples of the sieved soil were air-dried or stored at 5°C. The analyses reported in Table 25 were performed as described by Zantua and Bremner (1975).

Table 25. Analyses of soils.

Soil			Organic			
Series	Subgroup	pH	carbon	Sand	Silt	Clay
			---------- % ----------			
Canisteo	Typic Haplaquoll	7.7	3.0	30	39	31
Harps	Typic Calciaquoll	7.9	4.2	11	49	40
Storden	Typic Udorthent	8.1	1.2	57	24	19

Dalapon (Dowpon) was obtained from Dow Chemical U.S.A., Midland, MI. Atrazine (Aatrex) and simazine (Princep) were obtained from Ciba-Geigy Corp., Greensboro, NC. To study the effects of these herbicides on denitrification of nitrate in soil, samples of field-moist or air-dried soil containing 30 g of oven-dry material were placed in 1.2-L flasks fitted with standard taper (34/45) ground-glass joints and treated with 5 mL of H_2O containing 0, 0.15, 0.30, 0.75 or 3.00 mg of the herbicide under study, 5 mL of H_2O containing 9 mg of N as KNO_3 and 0 or 3 mg of

C as glucose and the amount of H_2O needed to bring the total volume of H_2O to 15 mL. Each flask was then sealed with a glass stopper fitted with a standard taper (34/45) ground-glass joint and a glass stopcock and was connected to a manifold system attached to a mercury (Hg) manometer. The flasks were evacuated via the stopcock and filled with He to slightly above atmospheric pressure, and this process was repeated three times. The flasks were then brought to atmospheric pressure with He and placed in an incubator at 30°C. The atmospheres in the flasks were subsequently sampled at intervals for determination of N_2O, NO, N_2 and O_2 by the GC-ultrasonic detector method described by Blackmer and Bremner (1977). The analyses for O_2 were performed to check that anaerobic conditions had been maintained. The results of analyses for NO are not reported because only trace amounts of this gas could be detected. All experiments were performed in triplicate.

Analyses of soil samples for nitrite were performed by the colorimetric procedure described by Bremner (1965). Analyses for nitrate were performed by the steam distillation method described by Bremner and Keeney (1966).

No loss of nitrate or production of nitrite, N_2O, NO or N_2 could be detected when soil samples previously sterilized by autoclaving at 121°C for 1 hour were incubated (30°C, 15 mL of H_2O) under He for 8 days after treatment with 9 mg of nitrate-N as KNO_3.

RESULTS AND DISCUSSION

None of the herbicides studied had a significant effect on denitrification of nitrate by soil microorganisms when applied at the rate of 5, 10, 25 or 100 μg g^{-1} soil to soils that were air-dried or kept field-moist before use. The data obtained using 100 μg of each herbicide g^{-1} soil are reported in Table 26. Dalapon has been reported to inhibit denitrification of NO_3^- in a flooded soil when applied at the rate of 10 μg g^{-1} soil (Weeraratna, 1980) and to inhibit denitrification in a salt marsh sediment when applied at rates as low as 10 μg mL^{-1} sediment (Grant and Payne, 1982). McElhannon et al. (1984) and Mills (1984) reported that atrazine and simazine inhibited denitrification in soil when these herbicides were applied at rates as low as 0.5 μg g^{-1} soil. As noted previously, Rolston and Cervelli (1980) reported that atrazine inhibited denitrification of nitrate in soil when applied at the rate of 28.5 μg g^{-1} soil. They subsequently reported, however, that atrazine stimulated nitrate reduction and inhibited reduction of N_2O to N_2 by denitrifying microorganisms when applied at the rate of 3 μg g^{-1} soil to a soil column, but did not affect reduction of NO_2^- to N_2O (Cervelli and Rolston, 1983).

The experiments reported in Table 26 involved incubation for 144 or 168 h. The influence of incubation time on the effects of dalapon, atrazine and simazine on denitrification in soil was studied because several of the researchers cited incubated for only 24 to 72 h (Grant and Payne, 1982; McElhannon et al., 1984; Mills, 1984). I found that, when

Table 26. Effects of 100 μg g^{-1} soil of dalapon, atrazine and simazine on denitrification of nitrate in soils[a]

Soil[b]	Herbicide	NO_3^--N lost	N produced			
			NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
		μg g^{-1} soil				
C (FM)	None	42	3	19	20	42
	Dalapon	41	2	19	21	42
	Atrazine	41	0	20	21	41
	Simazine	42	3	18	21	42
C (AD)	None	62	0	9	53	62
	Dalapon	61	0	13	49	62
	Atrazine	64	0	11	53	64
	Simazine	62	0	9	53	62
H (FM)	None	37	0	14	23	37
	Dalapon	38	0	15	23	38
	Atrazine	38	0	15	24	39
	Simazine	37	0	15	22	37
H (AD)	None	82	0	16	66	82
	Dalapon	81	0	22	60	82
	Atrazine	83	0	21	62	83
	Simazine	82	0	17	65	82
S (FM)	None	26	0	6	20	26
	Dalapon	27	0	7	21	28
	Atrazine	29	0	6	22	28
	Simazine	27	0	5	22	27
S (AD)	None	67	0	2	65	67
	Dalapon	66	0	1	65	66
	Atrazine	67	0	3	64	67
	Simazine	68	0	3	65	68

[a] Samples of field-moist (FM) or air-dried (AD) soil containing 30 g of oven-dry material were incubated (30°C, 15 mL of H_2O) under He for 6 (AD) or 7 (FM) days after treatment with 9 mg of nitrate-N as KNO_3 and 0 or 3 mg herbicide specified.

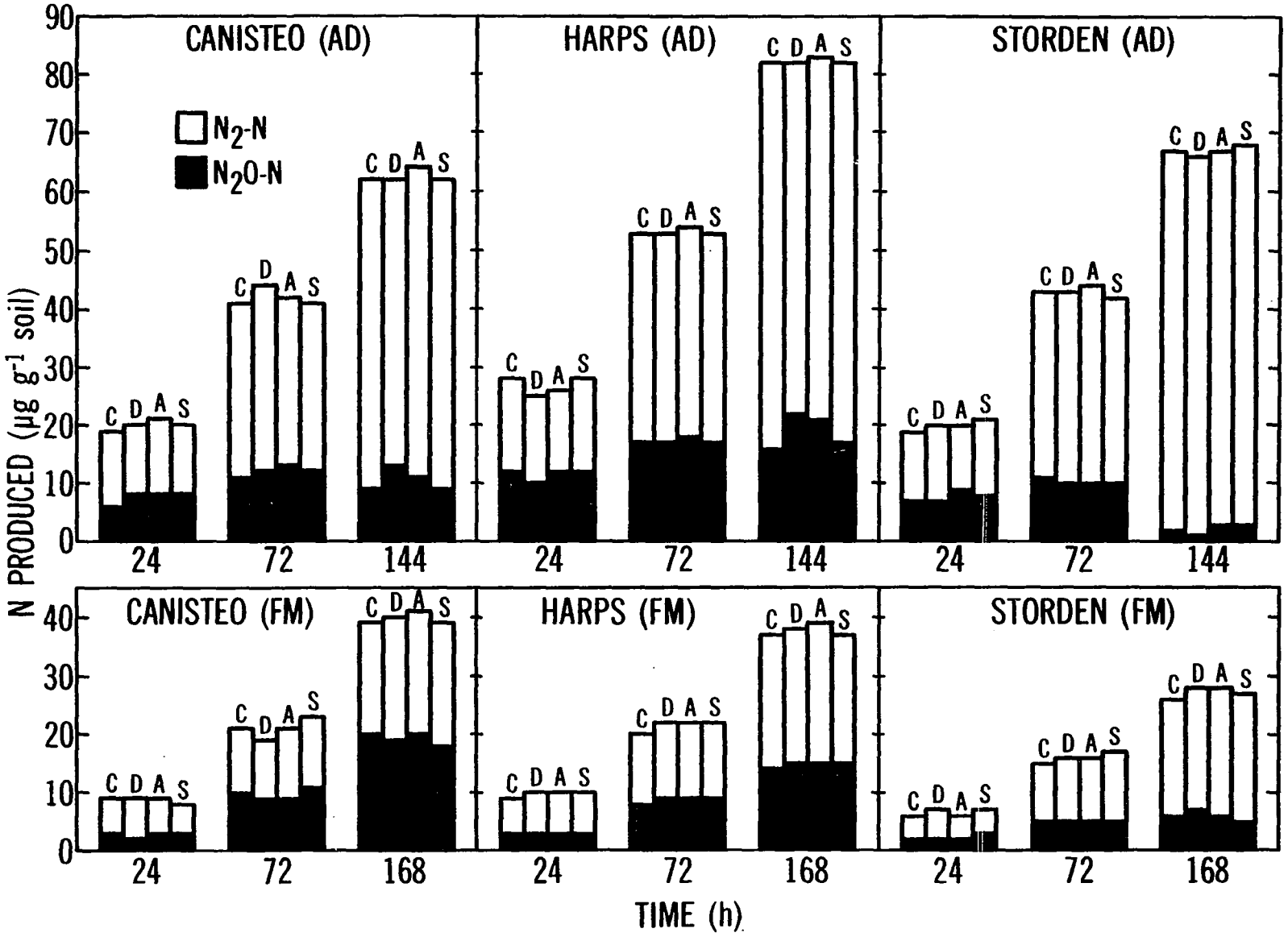
[b] C, Canisteo; H, Harps; S, Storden.

applied at the rate of 5, 10, 25 or 100 μg g^{-1} soil to soils that were air-dried or kept field-moist before use, none of the herbicides studied affected the production of N_2O or N_2 in soils incubated under anaerobic conditions for 24, 72, 144 and 168 h after treatment with nitrate. The data obtained using 100 μg of each herbicide g^{-1} soil are reported in Figure 1.

Grant and Payne (1982) reported that, when dalapon and atrazine were applied at the rate of 10 μg mL sediment, only dalapon inhibited N_2O production in 72 h by denitrification of nitrate in a salt marsh sediment. They did not, however, measure production of N_2 as well as N_2O, so their work did not permit any valid conclusion regarding the effects of dalapon and atrazine on denitrification. McElhannon et al. (1984) reported that, when applied at the rate of 2.0 μg mL^{-1} medium, both atrazine and simazine inhibited N_2O production in 72 h by denitrification of nitrate in a liquid medium inoculated with soil.

Table 27 shows the data obtained when the experiments reported in Table 26 were repeated using soils that had been amended with available organic carbon (as glucose) to promote denitrification of nitrate by soil microorganisms. The effect of adding available organic carbon was investigated because in several previous studies of the effects of dalapon, atrazine and simazine on denitrification, the herbicides were added to soil in an organic solvent (ethanol, acetone or methanol) that is readily utilized by soil microorganisms (Bollag and Henninger, 1976; Rolston and Cervelli, 1980; McElhannon et al., 1984) or were added to soil amended with available organic carbon (as glucose) (Rolston and

Figure 1. Effects of 100 μg g^{-1} soil of dalapon (D), atrazine (A) and simazine (S) on production of N_2O-N and N_2-N by denitrification of nitrate in soils that were air-dried (AD) or kept field-moist (FM) before use [C = control (no herbicide added)]


CANISTEO (AD)
HARPS (AD)
STORDEN (AD)
CANISTEO (FM)
HARPS (FM)
STORDEN (FM)
N2-N
N2O-N
N PRODUCED (µg g-1 soil)
TIME (h)
C D A S
24
72
144
168
0
10
20
30
40
50
60
70
80
90

Cervelli, 1980; Grant and Payne, 1982; McElhannon et al., 1984; Mills, 1984). I found that none of the herbicides in my study had an appreciable effect on denitrification of nitrate when applied at the rate of 5, 10, 25 or 100 μg g^{-1} soil to soils that were air-dried or kept field-moist before use and were amended with glucose before anaerobic incubation. The data obtained using 100 μg of each herbicide g^{-1} soil are reported in Table 27.

Previous work by Bollag and Henninger (1976) showed that atrazine and simazine had no effect on denitrification in soil when they were dissolved in ethanol and applied at the rate of 100 μg g^{-1} soil. Grant and Payne (1982) reported that, when applied at rates as high as 1000 μg mL^{-1} sediment, atrazine had no effect on denitrification in a salt marsh sediment amended with glucose. However, my finding that dalapon, atrazine and simazine had no effect on denitrification when applied at rates of 5, 10, 25 or 100 μg g^{-1} soil to soils that had been amended with available organic carbon is contrary to findings of Grant and Payne (1982), Rolston and Cervelli (1980), McElhannon et al. (1984) and Mills (1984). Grant and Payne (1982) reported that dalapon inhibited denitrification in a salt marsh sediment amended with glucose when it was applied at rates as low as 10 μg mL^{-1} sediment. Rolston and Cervelli (1980) reported that, when applied at the rate of 28.5 μg g^{-1} soil, atrazine inhibited denitrification in a soil amended with glucose, and McElhannon et al. (1984) and Mills (1984) reported that, when applied at the rate of 2.0 μg mL^{-1} medium, atrazine and simazine inhibited denitrification in a liquid medium inoculated with soil when they were

Table 27. Effects of 100 μg g^{-1} soil of dalapon, atrazine and simazine on denitrification of nitrate in soils amended with glucose[a]

Soil[b]	Herbicide	NO_3^--N lost	N produced			
			NO_2^--N	N_2O-N	N_2-N	(NO_2^-+N_2O+N_2)-N
		μg g^{-1} soil				
C (FM)	None	167	108	19	40	167
	Dalapon	166	106	20	40	166
	Atrazine	171	108	21	40	169
	Simazine	167	109	20	39	168
C (AD)	None	204	161	14	29	204
	Dalapon	205	164	13	28	205
	Atrazine	203	159	15	30	204
	Simazine	203	161	14	28	203
H (FM)	None	118	53	32	33	118
	Dalapon	119	55	28	35	118
	Atrazine	117	52	32	33	117
	Simazine	118	54	32	32	118
H (AD)	None	206	148	18	40	206
	Dalapon	207	148	20	38	206
	Atrazine	208	152	20	37	209
	Simazine	207	149	20	39	208
S (FM)	None	124	57	24	43	124
	Dalapon	127	60	23	44	127
	Atrazine	123	55	26	42	123
	Simazine	124	58	23	43	124
S (AD)	None	188	148	21	19	188
	Dalapon	189	148	20	21	189
	Atrazine	187	149	23	17	189
	Simazine	189	150	19	20	189

[a] Samples of field-moist (FM) or air-dried (AD) soil containing 30 g of oven-dry material were incubated (30°C, 15 mL of H_2O) under He for 1 (AD) or 3 (FM) days after treatment with 9 mg of nitrate-N as KNO_3, 3 mg of carbon as glucose and 0 or 3 mg herbicide specified.

[b] C, Canisteo; H, Harps; S, Storden.

added to the medium in methanol.

The only explanation I can suggest for the divergence between my findings and those of previous workers is that the analyses performed by these workers did not permit reliable conclusions concerning the effects of dalapon, atrazine and simazine on denitrification in soil. In the work reported here, I demonstrated that loss of nitrate-N by denitrification could be accounted for by production of (nitrite + N_2O + N_2)-N. Previous workers have not provided such evidence of the validity of their methodology.

In summary, the data obtained did not confirm recent reports that small amounts of dalapon, atrazine and simazine inhibit denitrification of nitrate in soil. No inhibition of denitrification was observed when these herbicides were applied at the rate of 5, 10, 25 or 100 $\mu g\ g^{-1}$ soil in the presence or absence of glucose.

SUMMARY

The influence of dalapon (2,2-dichloropropanoic acid), atrazine (6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine) and simazine (6-chloro-*N*,*N'*-diethyl-1,3,5-triazine-2,4-diamine) on denitrification of nitrate in soil was studied by determining the effects of different amounts of these herbicides on the amounts of nitrate lost and the amounts of nitrite, N_2O and N_2 produced when field-moist and air-dried samples of soil were incubated anaerobically after treatment with nitrate or with nitrate and glucose. The data obtained did not confirm recent reports that small amounts of these herbicides inhibit denitrification in soil. No inhibition of denitrification was observed when dalapon, atrazine and simazine were applied at the rate of 5, 10, 25 or 100 $\mu g\ g^{-1}$ soil in the presence or absence of glucose.

GENERAL SUMMARY

The influence of 14 urease inhibitors, 28 nitrification inhibitors and 33 pesticides on denitrification of nitrate in soil was studied by determining the effects of different amounts of these compounds on the amounts of nitrate lost and the amounts of nitrite, N_2O and N_2 produced when soil samples were incubated anaerobically with nitrate or with nitrate and organic carbon (as mannitol or glucose). The urease inhibitors used were catechol, hydroquinone, 1,4-benzoquinone, 2,5-dichloro-1,4-benzoquinone, 2,6-dichloro-1,4-benzoquinone, 2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, sodium-4-chloromercuribenzoate, phenylphosphorodiamidate, phosphoric triamide, *N*-phenylphosphoric triamide, *N*-(diaminophosphinyl)-benzeneacetamide, *N*-(diaminophosphinyl)benzamide and 4-fluoro-*N*-(diaminophosphinyl)benzamide. The nitrification inhibitors used were nitrapyrin (N-Serve, N-Serve 24, N-Serve 24E), potassium azide, 2-amino-4-chloro-6-methylpyrimidine, 2-mercaptobenzothiazole, sulfathiazole, etridiazole (Dwell), 4-amino-1,2,4-triazole, 3-mercapto-1,2,4-triazole, 2,4-diamino-6-trichloromethyl-*s*-triazine, potassium amylxanthate, potassium ethylxanthate, sodium ethylxanthate, sodium isopropylxanthate, guanylthiourea, thiourea, 2-chloroacetamide, 2-fluoroacetamide, 4-nitrobenzotrichloride, 4-mesylbenzotrichloride, caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, gallic acid, sodium thiocarbonate, sodium diethyldithiocarbamate, phenylmercuric acetate and dicyandiamide. The pesticides used included 20 herbicides

[butylate, EPTC, chlorpropham, propham, diuron, linuron, monuron, siduron, alachlor, trifluralin, 2,4-D amine, 2,4-D ester, atrazine, cyanazine, metribuzin, simazine, dalapon, chloramben, dicamba and dinoseb], seven insecticides [lindane, fenitrothion, fonofos, malathion, phorate, terbufos and carbofuran] and six fungicides [mancozeb, maneb, thiram, benomyl, captan and terrazole].

None of the urease inhibitors studied had a significant effect on denitrification when applied at the rate of 1 or 10 $\mu g\ g^{-1}$ soil. Three of the inhibitors (2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone and *N*-phenylphosphoric triamide) retarded denitrification when applied at the rate of 50 $\mu g\ g^{-1}$ soil, but most of the other urease inhibitors tested enhanced denitrification when applied at this rate. Four of the inhibitors (2,5-dimethyl-1,4-benzoquinone, 2,6-dimethyl-1,4-benzoquinone, sodium-4-chloromercuribenzoate and catechol) retarded denitrification when applied at the rate of 50 $\mu g\ g^{-1}$ soil to soils amended with mannitol.

Only one of the nitrification inhibitors studied (potassium azide) retarded denitrification when applied at the rate of 10 $\mu g\ g^{-1}$ soil, and only two (potassium azide and 2,4-diamino-6-trichloromethyl-*s*-triazine) inhibited denitrification when applied at the rate of 50 $\mu g\ g^{-1}$ soil. The other inhibitors either had no appreciable effect on denitrification, or enhanced denitrification, when applied at the rate of 10 or 50 $\mu g\ g^{-1}$ soil, enhancement being most marked with 3-mercapto-1,2,4-triazole. Seven of the nitrification inhibitors (potassium azide, sulfathiazole, potassium ethylxanthate, sodium isopropylxanthate,

4-nitrobenzotrichloride, sodium thiocarbonate and phenylmercuric acetate) retarded denitrification when applied at the rate of 50 μg g^{-1} soil to soil that had been amended with mannitol to promote microbial activity.

Reports that nitrapyrin (N-Serve) and etridiazole (Dwell) inhibit denitrification in soil when applied at rates as low as 0.5 μg g^{-1} soil could not be confirmed. No inhibition of denitrification was observed when these compounds were applied at the rate of 10 μg g^{-1} soil, and enhancement of denitrification was observed when they were applied at the rate of 50 or 100 μg g^{-1} soil.

None of the herbicides studied significantly affected denitrification of nitrate when applied at the rate of 10 μg g^{-1} soil, but dinoseb increased the ratio of N_2/N_2O in the gaseous products of denitrification when applied at this rate. Butylate, EPTC, diuron, atrazine, simazine and dalapon had no significant effect on denitrification when applied at the rate of 50 μg g^{-1} soil, whereas metribuzin and dinoseb enhanced denitrification when applied at this rate. The influence of the other herbicides on denitrification depended on the soil, but all enhanced or inhibited denitrification in at least one soil.

Recent reports that small amounts of dalapon, atrazine and simazine inhibit denitrification in soil could not be confirmed. No inhibition of denitrification was observed when these herbicides were applied at the rate of 5, 10, 25 or 100 μg g^{-1} soil in the presence or absence of glucose.

None of the insecticides studied had a significant effect on

denitrification when applied at the rate of 10 μg g^{-1} soil. When applied at the rate of 50 μg g^{-1} soil, lindane, fonofos and malathion enhanced denitrification in the three soils studied, whereas fenitrothion, phorate, terbufos and carbofuran either had no appreciable effect on denitrification in these soils or enhanced denitrification in at least one of the soils.

None of the fungicides studied had an appreciable effect on denitrification when applied at the rate of 10 μg g^{-1} soil, but thiram increased the ratio of N_2/N_2O in the gaseous products of denitrification. Captan inhibited denitrification in two of the three soils studied when applied at the rate of 50 μg g^{-1} soil. The other five fungicides either had no significant effect on denitrification, or enhanced denitrification, when applied at this rate. Reports that small amounts of maneb, thiram and terrazole inhibit denitrification in soil could not be confirmed.

LITERATURE CITED

Ashworth J., Akerboom H. M. and Crepin J. M. 1980. Inhibition by xanthates of nitrification and urea hydrolysis in soil. Soil Science Society of America Journal 44: 1247-1249.

Ashworth J., Briggs G. G., Evans A. A. and Matula J. 1977. Inhibition of nitrification by nitrapyrin, carbon disulfide, and trithiocarbonate. Journal of Science of Food and Agriculture 28: 673-683.

Atkinson G. 1973. Effects of diquat on the microbiological organisms in the soil of lakes. Proceeding of the Pollution Research Conference, Wairakei, New Zealand. DSIR Information Series Number 97: 529-538.

Atlas R. M., Pramer D. and Bartha R. 1978. Assessment of pesticide effects on non-target soil microorganisms. Soil Biology and Biochemistry 10: 231-239.

Beste C. E., Chairman. 1983. Herbicide Handbook of the Weed Science Society of America, 5th edition. Weed Science Society of America, Champaign, Illinois.

Blackmer A. M. and Bremner J. M. 1977. Gas chromatographic analysis of soil atmospheres. Soil Science Society of America Journal 41: 908-912.

Bollag J. M. and Henninger N. M. 1976. Influence of pesticides on denitrification in soil and with an isolated bacterium. Journal of Environmental Quality 5: 15-18.

Bollag J. M. and Kurek E. J. 1980. Nitrite and nitrous oxide accumulation during denitrification in the presence of pesticide derivatives. Applied and Environmental Microbiology 39: 845-849.

Bollag J. M. and Nash C. L. 1974. Effect of chemical structure of phenylureas and anilines on the denitrification process. Bulletin of Environmental Contamination and Toxicology 12: 241-248.

Bremner J. M. 1965. Inorganic forms of nitrogen. p. 1179-1237. *In* C. A. Black et al., Eds. Methods of Soil Analysis. Monograph 9. Volume 2. American Society of Agronomy, Madison, Wisconsin.

Bremner J. M. and Blackmer A. M. 1980. Mechanisms of nitrous oxide production in soils. p.279-291. *In* P. A. Trudinger et al., Eds. Biochemistry of Ancient and Modern Environments. Australian Academy of Science, Canberra, Australia.

Bremner J. M. and Bundy L. G. 1976. Effects of potassium azide on transformations of urea nitrogen in soils. Soil Biology and Biochemistry 8: 131-133.

Bremner J. M. and Douglas L. A. 1971. Inhibition of urease activity in soils. Soil Biology and Biochemistry 3: 297-307.

Bremner J. M. and Douglas L. A. 1973. Effects of some urease inhibitors on urea hydrolysis in soils. Soil Science Society of America Proceedings 37: 225-226.

Bremner J. M. and Hauck R. D. 1974. Perspectives in soil and fertilizer nitrogen research. International Congress of Soil Science, 10th Transactions, Moscow. 9: 13-27.

Bremner J. M. and Keeney D. R. 1966. Determination and isotope-ratio analysis of different forms of nitrogen in soils. 3. Exchangeable ammonium, nitrate, and nitrite by extraction-distillation methods. Soil Science Society of America Proceedings 30: 577-582.

Bundy L. G. and Bremner J. M. 1973a. Effects of substituted *p*-benzoquinones on urease activity in soils. Soil Biology and Biochemistry 5: 847-853.

Bundy L. G. and Bremner J. M. 1973b. Inhibition of nitrification in soils. Soil Science Society of America Proceedings 37: 396-398.

Bundy L. G. and Bremner J. M. 1974a. Effects of urease inhibitors on nitrification in soils. Soil Biology and Biochemistry 6: 27-30.

Bundy L. G. and Bremner J. M. 1974b. Effects of nitrification inhibitors on transformations of urea nitrogen in soils. Soil Biology and Biochemistry 6: 369-376.

Byrnes B. H., Savant N. K. and Craswell E. T. 1983. Effect of a urease inhibitor phenylphosphorodiamidate on the efficiency of urea applied to rice. Soil Science Society of America Journal 47: 270-274.

Cervelli S. and Rolston D. E. 1983. Influence of atrazine on denitrification in soil columns. Journal of Environmental Quality 12: 482-486.

Cribbs W. H. and Mills H. A. 1979. Influence of nitrapyrin on the evolution of N_2O from an organic medium with and without plants. Communications in Soil Science and Plant Analysis 10: 785-794.

De B. K. and Mukhopadhyay S. 1971. Effect of MCPA and Stam F-34 on the occurrence of some nutritional groups of bacteria in the rice fields of West Bengal. International Rice Commission Newsletter 20(3): 35-40.

Endo T., Kusaka T., Tan M. and Sakai M. 1982. Effect of the insecticide cartap hydrochloride on soil microflora. Journal of Pesticide Science 7: 1-7.

Frankenberger W. T. Jr. and Tabatabai M. A. 1982. Transformations of amide nitrogen in soils. Soil Science Society of America Journal 46: 280-284.

Goring C. A. I. 1962a. Control of nitrification by 2-chloro-6-(trichloromethyl)pyridine. Soil Science 93: 211-218.

Goring C. A. I. 1962b. Control of nitrification of ammonium fertilizers and urea by 2-chloro-6-(trichloromethyl)pyridine. Soil Science 93: 431-439.

Goring C. A. I. and Laskowski D. A. 1982. The effects of pesticides on nitrogen transformations in soils. p. 689-720. *In* F. J. Stevenson, Ed. Nitrogen in Agricultural Soils. Monograph No. 22. American Society of Agronomy, Madison, Wisconsin.

Grant M. A. and Payne W. J. 1982. Effects of pesticides on denitrifying activity in salt marsh sediments. Journal of Environmental Quality 11: 369-372.

Hart L. T. and Larson A. D. 1966. Effect of 2,4-dichlorophenoxyacetic acid on different metabolic types of bacteria. Bulletin of Environmental Contamination and Toxicology 1: 108-120.

Hauck R. D. 1972. Synthetic slow-release fertilizers and fertilizer amendments. p. 633-690. *In* C. A. I. Goring and J. W. Hamaker, Eds. Organic Chemicals in the Soil Environment, Volume 2. Marcel Dekker, New York.

Hauck R. D. 1980. Mode of action of nitrification inhibitors. p. 19-32. *In* M. Stelly et al., Eds. Nitrification Inhibitors - Potentials and Limitations. ASA Special Publication Number 38. American Society of Agronomy, Madison, Wisconsin.

Hauck R. D. 1984. Technological approaches to improving the efficiency of nitrogen fertilizer use by crop plants. p. 551-560. *In* R. D. Hauck et al., Eds. Nitrogen in Crop Production. American Society of Agronomy, Madison, Wisconsin.

Held P., Lang S., Tradler E., Klepel M., Drohne D., Hartbrich H. J., Rothe G., Scheler H., Grundmeier S. and Trautmann A. 1976. Agent for reducing the loss of plant-available nitrogen in cultivated soil. East German Patent Number 122,177. Chemical Abstracts 87: 67315w.

Henninger N. M. and Bollag J. M. 1976. Effect of chemicals used as nitrification inhibitors on the denitrification process. Canadian Journal of Microbiology 22: 668-672.

Heytler P. G., Davidson S. H. and Hardy R. W. F. 1984. Improved inhibitors of soil nitrification. p. 107. Agronomy Abstracts. American Society of Agronomy, Madison, Wisconsin.

Hooper A. B. and Terry K. R. 1973. Specific inhibitors of ammonia oxidation in *Nitrosomonas*. Journal of Bacteriology 115: 480-485.

Hughes T. D. and Welch L. F. 1970. Potassium azide as a nitrification inhibitor. Agronomy Journal 62: 595-599.

Ishizawa S. and Matsuguchi T. 1966. Effects of pesticides and herbicides upon microorganisms in soil and water under waterlogged conditions. Bulletin of the National Institute of Agricultural Sciences, Japan, Series B 16: 1-90.

Lees H. 1952. The biochemistry of nitrifying organisms. I. The ammonia-oxidizing systems of *Nitrosomonas*. Biochemical Journal 52: 134-139.

Lodhi M. A. K. 1982. Additional evidence of inhibition of nitrifiers and possible cycling of inhibitors produced by selected plants in a climax community. Bulletin of the Torrey Botanical Club 109: 199-204.

Malzer G. L. 1979. Progress with nitrification inhibitors. Down to Earth 35(3): 1-3.

Martens D. A. and Bremner J. M. 1984. Effectiveness of phosphoroamides for retardation of urea hydrolysis in soils. Soil Science Society of America Journal 48: 302-305.

Matsubara T. and Mori T. 1968. Studies on denitrification. IX. Nitrous oxide, its production and reduction to nitrogen. The Journal of Biochemistry 64: 863-871.

Matzel W., Lippold H. and Heber R. 1979. Nitrogen uptake and balance of the fertilizers urea, urea with urease inhibitor, and ammonium nitrate applied to spring wheat at stem elongation growth stage. p. 67-81. *In* Isotopes and Radiation in Research on Soil-Plant Relationships. International Atomic Energy Agency, Vienna.

McElhannon W. S. and Mills H. A. 1981a. Inhibition of denitrification by nitrapyrin with field-grown sweet corn. Journal of the American Society for Horticulture Science 106: 673-677.

McElhannon W. S. and Mills H. A. 1981b. Suppression of denitrification with nitrapyrin. HortScience 16: 530-531.

McElhannon W. S., Mills H. A. and Bush P. B. 1984. Simazine and atrazine - suppression of denitrification. HortScience 19: 218-219.

Mills H. A. 1984. A procedure for rapidly evaluating the effect of chemicals on denitrification. Communications in Soil Science and Plant Analysis 15: 1007-1016.

Mills H. A., Barker A. V. and Maynard D. N. 1976. Nitrate accumulation in radish as affected by nitrapyrin. Agronomy Journal 68: 13-17.

Mills H. A. and McElhannon W. S. 1983. Effect of broadcast vs. banded applications of nitrapyrin on yield and nitrogen concentration in field-grown sweet corn. HortScience 18: 740-741.

Mills H. A. and McElhannon W. S. 1984. Terrazole suppression of denitrification. HortScience 19: 54-55.

Mills H. A. and Pokorny F. A. 1978. The influence of nitrapyrin on N retention and tomato growth in sand-bark media. Journal of the American Society for Horticultural Science 103: 662-664.

Mitsui S., Watanabe I., Honma M. and Honda S. 1964. The effect of pesticides on the denitrification in paddy soil. Soil Science and Plant Nutrition 10: 107-115.

Notton B. A., Watson E. F. and Hewitt E. J. 1979. Effects of N-Serve [2-chloro-6-(trichloromethyl)pyridine] formulations on nitrification and on loss of nitrate in sand culture experiments. Plant and Soil 51: 1-12.

Parr J. F., Carroll B. R. and Smith S. 1971. Nitrification inhibition in soils: I. A comparison of 2-chloro-6-(trichloromethyl)pyridine and potassium azide formulated with anhydrous ammonia. Soil Science Society of America Proceedings 35: 469-473.

Reddy G. R. 1964. Effect of mixing varying quantities of dicyandiamide with ammonium fertilizers on nitrification of ammonia in soils. Canadian Journal of Soil Science 44: 254-259.

Rice E. L. and Pancholy S. K. 1973. Inhibition of nitrification by climax ecosystems. II. Additional evidence and possible role of tannins. American Journal of Botany 60: 691-702.

Rice E. L. and Pancholy S. K. 1974. Inhibition of nitrification by climax ecosystems. III. Inhibitors other than tannins. American Journal of Botany 61: 1095-1103.

Rodgers G. A., Widdowson F. V., Penny A. and Hewitt M. V. 1983. Effects of several nitrification inhibitors, when injected with aqueous urea, on yields and nitrogen recoveries of ryegrass leys. Journal of Agricultural Science Cambridge 101: 637-656.

Rolston D. E. and Cervelli S. 1980. Denitrification as affected by irrigation frequency and applied herbicides. p. 189-199. *In* Agrochemical Residue-biota Interactions in Soil and Aquatic Ecosystems. International Atomic Energy Agency, Vienna.

Sandhu M. S. and Moraghan J. T. 1972. Influence of three chemicals on soil biological activity. Communications in Soil Science and Plant Analysis 3: 439-447.

Sethunathan N. 1970. Foliar sprays of growth regulators and rhizosphere effects in *Cajanus cajan* Millsp. II. Qualitative changes in the rhizosphere and certain metabolic changes in the plant. Plant and Soil 33: 71-80.

Sommer K. 1972. Status and accomplishment of agrichemical and agribiological research. XXIII. Nitrificides. Part II. Ammonium nitrificides of U.S. and Japanese origin. Landwirtschftliche Forschung, Sonderheft 27: 74-82. Chemical Abstracts 78: 3261g.

Torstensson L. 1974. Effects of MCPA, 2,4,5-T, linuron, and simazine on some functional groups of soil microorganisms. Swedish Journal of Agricultural Research 4: 151-160.

Varsa E. C. and Huber D. M. 1983. Nitrogen inhibitors worth a look. Solutions 27(2): 60-64.

Vlek P. L. G., Stumpe J. M. and Byrnes B. H. 1980. Urease activity and inhibition in flooded soil systems. Fertilizer Research 1: 191-202.

Weeraratna C. S. 1980. Effect of dalapon-sodium on nitrification and denitrification in a tropical loam soil. Weed Research 20: 291-293.

Zantua M. I. and Bremner J. M. 1975. Comparison of methods of assaying urease activity in soils. Soil Biology and Biochemistry 7: 291-295.

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